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ANATOMY OF THE POTATO PLANT, WITH SPECIAL REFERENCE TO THE ONTOGENY OF THE VASCULAR SYSTEM[1]

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INTRODUCTION

Recent studies of potato diseases have made it obvious that more accurate knowledge regarding the normal structure of potato plants (*Solanum tuberosum*) is necessary. The present work was undertaken in order to obtain data which may make possible a more rapid progress in the investigation of several important potato diseases, such as the potato leafroll. This disease, which is a serious one, was the main reason for undertaking the present detail study. It was the original intention of the writer to investigate the pathological anatomy of plants suffering from leafroll, but because of the lack of adequate information regarding their normal structure no information for comparative purposes was available.

The anatomy and development of the potato plant have been studied up to this time chiefly from the viewpoint of economic botany; consequently previous work relates largely to observations on gross morphology. A study of the origin, differentiation, and organization of the vascular supply, however, is essential to a clear understanding of the physiology of the organs and of the relation of this supply to tuber formation. Furthermore, such a study enables us to distinguish the significant points in all pathological changes.

The critical study of the internal anatomy of plants began only in the middle of the last century, when the work of Schleiden and other investigators raised botany to the level of other sciences. Schleiden, in his

[1] This work was begun in the field laboratory of the Office of Cotton, Truck, and Forage Crop Disease Investigations at Greeley, Colo., in the summer of 1916, and was continued in the Department of Plant Pathology at Cornell University under the direction of Prof. H. H. Whetzel and Dr. H. A. Edson, to whom the author wishes to express his gratitude for their courtesy and helpful suggestions. To Dr. A. J. Eames, of the Department of Botany, Cornell University, the writer is especially indebted for the constant advice and criticism received in preparation of materials, interpretation of slides, and editing of the paper. Thanks are also due to Mr. W. R. Fisher for accurate and painstaking work in the preparation of the photographs.

"Cell Theory," clearly set forth the relation between cells, tissues, and organs, and also treated in some detail the origin and function of each. Other investigators, stimulated by his work, added new facts and corrected the old errors, until finally a fairly clear understanding of the general internal anatomy of plants was gained. As may be expected, much of this earlier work relates to the xylem, as this tissue is by far the most easily distinguished under low magnification. The phloem remained a mystery up to the time of Hartig. This investigator reports and describes phloem elements, but it remained for Von Mohl to give a clear conception of the sieve tube and to show its significance as a conducting unit for plastic materials. Von Mohl, and later other investigators, reported the occurrence of sieve tubes in numerous plant families, but Hanstein (*2*)[1] was first to report their occurrence in the Solanaceae, the family to which the potato belongs.

Vesque (*5*) gives a short discussion on the distribution of the external and internal phloem in the Solanaceae. He also reports the occurrence of phloem fibers, but states that they are absent in the internal phloem when they are wanting in the external region.

Petersen (*9*) confirms Vesque and slightly extends his observations on the distribution and the relative amount of external and internal phloem in the different genera of the Solanaceae.

The first detailed discussion of the histology of the Solanaceae is given by Weiss (*10*). He writes as follows:

> The internal phloem groups always accompany the leaf-traces into the leaf and are differentiated only a little earlier than are the groups of external phloem. A distinct cambium is never developed between internal phloem and xylem. The internal phloem groups remain distinct and only in the smaller veins of the leaf blade do they unite with the external phloem. On the other hand, the internal phloem may be considered as derived from the external phloem, a theory which would explain the presence in the pith of fibers characteristic of the external phloem.

In 1872 Jurgens (*4*) published a thesis on the anatomy and physiology of the potato tuber in which he gives a general yet comprehensive account of the histological structure and development of the tuber as well as of the plant itself. Unlike other investigators of his time, he did not believe that the periderm is formed from the epidermis, but from the subepidermal layer, the original epidermis having become sloughed off.

The work of Schacht (*1*) is of interest only on account of its numerous and beautiful plates illustrating the internal anatomy and the morphology of the plant and the tuber.

De Vries, after publishing two papers, one on the development and germination of the potato tuber (*7*), the other on the seed (*6*), reported his researches on the anatomy and the physiology of the potato plant in the "Landwirtschaftliche Jahrbücher" (*8*). This paper is of considerable importance, especially for the physiologists. In fact, it is the only

[1] Reference is made by number (italic) to "Literature cited," pp. 251–252.

paper treating of the physiology of the potato plant in detail and in all aspects. The gross morphology, the anatomy of the organs and tissues, as well as the development of the leaf, are given careful consideration. However, valuable as is this paper, the author tells us nothing definite of the ontogeny of the vascular system or of the relative amount and relations of the different elements of the phloem and xylem. Moreover, since this work was written, our conception of the origin of the stele and of its relation to the leaf traces has undergone a radical change, so that, so far as histology is concerned, the work is out of harmony with present ideas. This deficiency in De Vries's work and the absolute lack of other reliable study make an investigation of the anatomy of the potato plant imperative, the more so since the recent studies of potato diseases require a clear conception of normal structures as a background for the investigation of the changes brought about by pathological conditions. With the purpose of meeting this need, the present study was undertaken. Here an attempt is made to clear up the points left in doubt by earlier workers. In addition new facts are given which may make possible a decision between the divergent views of present experimenters who have been working on the physiological importance of pathological changes. Several potato diseases have been studied with reference to such changes but of special interest and importance at the present time is the leafroll disease, which is causing a serious loss both in this country and in Europe. A discussion of these divergent views, together with new investigations on the subject of the leafroll disease, will be given in a later paper; the present contribution is concerned with the anatomy of the normal plant.

MATERIAL AND METHODS OF EXPERIMENTATION

The material for study was obtained both from plants grown in the greenhouse and in the disease garden of the Department of Plant Pathology of Cornell University. The Irish Cobbler variety furnished the material for investigation; other varieties, such as Early Rose, New York Rural, and Green Mountain were used for comparative study. Several fixing fluids were used, including those of Flemming; but the best results were obtained by the common chromacetic-acid fixer, 1 per cent chromic acid and 1 per cent acetic acid. The usual methods of dehydrating and embedding in paraffin were employed. Leaf sections were cut 5 μ thick, stem sections 7 to 12 μ thick, and stained with Haidenhain's iron alum hematoxylin and safranin.

GROSS MORPHOLOGY

Solanum tuberosum L. is an annual herbaceous dicotyledon, a member of the Solanaceae. In habit it is more or less spreading, but grows to a height of 2 to 5 feet. This habit form is constant, though selection, culture, and breeding have often brought about so great a variety of

flower color, of color and form of stem, of leaf and tuber that some varieties would hardly be recognized as members of the wild species. This is especially true of certain South American varieties recently introduced into this country.

The aerial stem of the potato plant is herbaceous and erect when young; in age it becomes spreading. It is glabrous and three-cornered with the margins drawn out in the upper part to form ribs or wings which are especially prominent in young plants. Older specimens often lose these winglike borders; the subterranean part of the stem lacks them entirely. The latter is terete and differs further from the aerial portion in lack of chlorophyll and of dermal appendages. Stomata are present but relatively few in number. The leaves of this part of the stem are small and scalelike. In the axils of these, stolons arise. The tubers are swellings of the ends of these stolons.

The potato plant grown from tubers has no taproot; the roots are fine and fibrous, arising usually in groups of three just above certain nodes of the subterranean portion of the stem. These roots penetrate the soil to a depth of 3 or 4 feet and often extend horizontally 2 feet from the plant. Although the amount of root development is great, the relative weight as that compared with the aerial portion is very low. According to Hosäus (3), the proportions between root and stem in the potato is 1 to 44, while in other plants it is rarely less than 1 to 10.

The leaves are arranged on the stem in a spiral with the divergence of $\frac{5}{13}$. The type of spiral is usually left, though numerous specimens with right spirals are found in plants raised both from seeds and from tubers. This is in agreement with a statement of De Vries (7), who found that the spiral of the eyes of the potato tuber may be either right or left, differing with the individual and with the variety.

The petiole is semicircular in cross section. The adaxial side is slightly concave, the abaxial side strongly convex. The petiole becomes flattened toward the base, and there sheathes nearly one-third of the circumference of the stem. The winglike ribs or edges of the petiole are decurrent on the stem unequally, one extending for one internode, the other for two. The leaves are irregularly pinnate; the leaflets are more or less petioled, and between the larger leaflets supplementary leaflets occur. The number of these is not constant, but usually between two pairs of large leaflets one or two smaller pairs are found. The leaflets are oval in outline, with margins entire or sometimes serrate. There is great variation in the length of the petiole, the petiolules, and in the number of leaflets, a condition resulting in a loose or a crowded appearance. These characters are usually constant and characteristic of certain varieties.

The venation of the leaves is of the netted type. From a strongly developed midrib lateral branches arise which anastomose freely, forming a dense reticulum. The course of the lateral veins is acrodromous, giving

to the margin of the leaves a greater density than has the inner part of the lamina.

Young potato leaves are densely clothed with hairs, some of which are long and straight, others short and of the glandular type. The straight hairs are either one or several celled. The glandular hairs have a spherical head, usually four-celled and borne upon a slender, short pedicel. The mature leaf, however, is only sparingly covered with hairs, and these arise mainly from the midrib and the lateral veins. Stomata are found on both surfaces, but are more abundant on the lower.

The inflorescence is a monochasial cyme. The peduncle, though lateral, occupies a central position, having become stronger in its development than the stem tip, pushing aside the latter, which comes to occupy a position apparently lateral. The flowers are 5-merous and are borne on bractless pedicels. The gamopetalous corolla is tubular, with five lobes which are white, yellow, purple, or blue in color. The calyx is also tubular and 5-lobed. There is a single whorl of five stamens alternating with the corolla lobes and attached to the tube. The stamens are straight or slightly curved, with yellow anthers which are longer than the filaments, and converge around the style, each opening by two pores at the top. There are two completely fused carpels, forming a 2-celled ovary with a single style and stigma. The ovary is superior; each cell has one axile placenta with numerous ovules, foliar in origin. The fruit "potato-apple" or "potato-ball" is a 2-celled, many-seeded berry, spherical or ovoid, and green or purplish in color. The seeds are small and kidney-shaped and are embedded in the green pulp of the fruit.

The flowers are homogenous and are self-pollinated. They produce no nectar and are rarely visited by insects. In many varieties the flowers do not open at all, but soon wither and drop off. Most of the pollen is sterile. The potent grains differ from the sterile ones in being smaller and more regular in shape.

ANATOMY

It seems best, before beginning a discussion of ontogeny, to give in detail the anatomy of the partly mature plant, chiefly because it facilitates the understanding of the early developmental history which at best is involved.

THE STEM

A cross section of a stem, at a stage when the leaves are nearly mature, shows (Pl. 27, A) a circle of fibrovascular bundles, limited on the inside by a well-developed pith, on the outside by the endodermis and cortex. The pith is of uniform nature, but the cortex possesses near the periphery a layer of collenchyma of rather uniform width, as seen in Plate 29, A. Plate 29, D, illustrates the stem epidermis, which is one cell thick and separated from the collenchyma by a subepidermal layer also one cell thick.

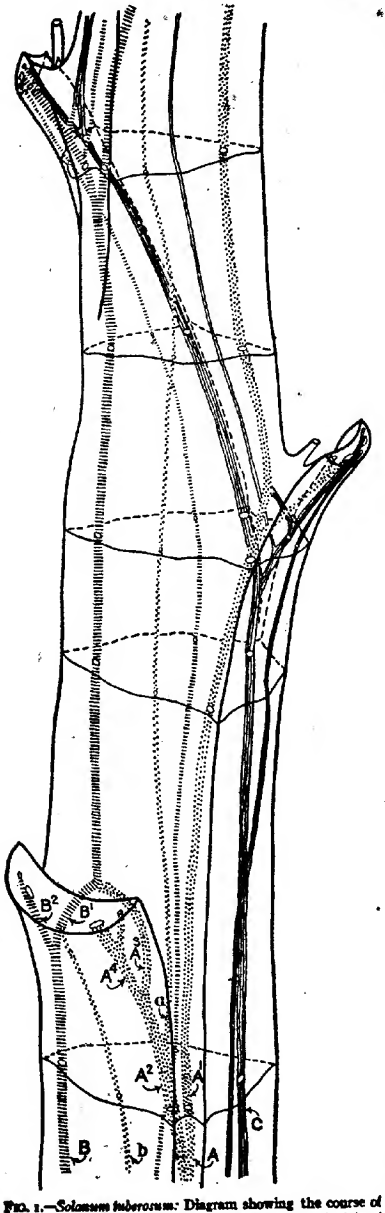

FIG. 1.—*Solanum tuberosum*: Diagram showing the course of the vascular bundles in the stem and the mode of origin of the vascular supply of the leaves (longitudinal). (See p. 227.)

The vascular cylinder is not of uniform width throughout; projections into the pith occur in places (Pl. 41, B). Its bundles are in part free, in part united by interfascicular cambium. The three corners of the stem are occupied each by a large bundle, and between each two of these a smaller bundle is found (Pl. 27, A). The vascular tissue follows naturally a longitudinal course in the stem, and its arrangement is closely related to the scheme of phyllotaxis. In traversing the stem in a vertical direction these bundles always remain approximately at the same distance from the center, entering the leaf as a whole or in part. Those bundles or strands of vascular tissue which pass out into a leaf are known as leaf-trace bundles. The relation of these to the stem is of much importance, since each leaf trace not only supplies the leaf to which it belongs with water and minerals, but also translocates the plastic materials manufactured in the leaf. The diagram (text figure 1) and the figures of Plates 27 and 28 show the course and origin of the various traces and their relation to each other and to the stem. A study of the petiole of the leaf shows that the vascular tissue, which occupies a semicircular area, consists of five groups: Three large groups and two smaller ones, the latter lying

in the outer corners (Pl. 43, C). These five groups are distinct traces, and their origin must be established and their course followed. A series of cross sections through as many internodes as are traversed by the longest trace is required for this task. Since the scheme of phyllotaxis has a divergence of 5/13, it may be supposed that the greatest extent of any of these traces would not exceed three internodes; this is actually the case.

As stated above, there are three large and three small stem bundles in the internodal region (Pl. 27, A). Below the node (base of diagram, figure 1, which illustrates a right spiral), where the stem begins to lose its three-cornered appearance and takes on the quadrangular outline, main group A begins to widen and breaks up into two more or less equal portions, A^1 and A^2. A little higher up, main group B also begins to widen. Immediately below the node, A^2 gives off a small bundle, a, which soon separates from the parent group and comes to lie between A^1 and A^2. (See also Pl. 27, B, a.) Just at the node, groups B and A^2 split, each forming two groups of nearly equal size: B gives rise to B^1 and B^2, and A^2 gives rise to A^3 and A^4. B^2 and A^4, given off by B and A^2, respectively, then divide unequally once more. These four last-mentioned groups pass out into the petiole forming the lateral traces there. The small stem group b, occupying originally the position between A and B, also passes out, but without division, into the petiole where it forms the median trace of the leaf. We see then that the vascular tissue of the petiole is derived from two chief sources:

(1) One of the smaller bundles of the stem, which becomes the median trace of the leaf.

(2) Branches of the two large stem groups A and B, which form the four lateral traces. The traces of the petiolar wings are derived from the lateral groups B^2 and A^4 after these have become separated from the stem bundles.

At the place of first branching of A, A^1 continues the original course of A. At the place of second branching those portions (B^1 and A^3) of B and A^2 which do not enter the petiole unite just above the insertion of the leaf to form a new stem bundle. Through this fusion the stem becomes three-cornered again.

The stem group A supplies two lateral traces of the petiole, gives rise to a new stem group A^1, and also to a new median trace, a, which becomes the median trace of the third leaf above; it further gives off some of its vascular tissue to B^1. B also supplies two lateral traces to the petiole, but does not form a third bundle; on the contrary, it receives vascular tissue from A^3 in compensation for the loss.

Bundle A^1 ascends for one internode and then forks again, but B^1 ascends unchanged for two internodes, then forks and gives rise to the same number of groups as B gave off near the first node, becoming the B of the second node. Bundle C similarly becomes the A of the second

node. This behavior of the bundles continues uniformly at succeeding nodes.

To summarize:

(1) The median trace ascends without fusion or forking through three internodes, and then passes into the petiole without branching.

(2) The lateral traces are given off at the node from two of the three large stem groups.

(3) Each large stem bundle ascends without branching in turn for two internodes and for one internode.

(4) Where a large stem bundle ascends for but one internode without branching, it divides three times, giving rise upon the first division to a new stem bundle, upon the second division to a new median trace, and upon the third division to lateral traces of the petiole. Upon the division in the node, half of the tissue given off to unite with that from the left (in a right spiral) adjacent group, forming a new large bundle directly above the insertion of the leaf. Where the bundle ascends for two internodes without branching, it gives off vascular tissue only for lateral traces of the petiole.

(5) Each leaf derives its supply from two large bundles and the smaller one lying between these. The method of derivation is uniform for all leaves, the bundles taking part being each time a different pair from those supplying the leaf below. In a right spiral the right member of the set supplying a leaf supplies also the leaf above, becoming there the left member of the set. The median trace of a given leaf is formed just below the third node below the leaf it supplies.

The vascular tissue shows bicollateral arrangement of its elements, a condition most clearly seen in the larger bundles of the stem. An examination of Plate 29, A, shows at first glance a wedge-shaped mass of rather large, dark-staining cells, the xylem. At more or less equal distances to the outside and the inside of the xylem are small groups of thin-walled cells which make up the phloem. The external phloem is separated from the xylem by a layer of uniform, rectangular cells, the cambium. The internal phloem is also separated from the xylem, but in this case by thin-walled, irregular cells (Pl. 29, C). These cells are much smaller than those of the pith and form what may be called the perimedullary zone of the vascular cylinder (stele). The external phloem groups are separated from one another and from the endodermis by parenchymatous cells of irregular size, which together constitute the pericyclic region of the stele (Pl. 29, A, B). The internal phloem abuts directly on the pith, and many of its groups are completely surrounded by pith cells. The phloem in both regions is made up of cell groups which in the outer zone are small and form a more or less continuous band and which in the inner region are variable in size and more scattered (Pl. 29, C).

A close examination of the xylem (Pl. 42, A) shows that the larger outer cells are arranged somewhat in radial rows, in which the individual cells either abut on one another or are separated by small thin-walled parenchyma cells. The walls of these elements are scalariform, reticulate, or pitted (Pl. 32, C; 33, E). Occupying the tip of the wedge-shaped mass are smaller xylem elements scattered among parenchyma cells. These have secondary thickenings in the form of rings or spirals and are the elements which mature before elongation ceases, the so-called protoxylem (Pl. 29, A, C; 42, C). Frequently these protoxylem elements appear crushed (Pl. 29, C), and show only part of the secondary thickening of the wall—that is, half a ring or part of a spiral band. The larger elements external to these cells make up the metaxylem. Though the arrangement of the xylem cells is somewhat irregular, the smallest, oldest, elements are found adjacent to the pith, showing that the order of development is from within out or centrifugal, and that the arrangement of these cells is therefore endarch.

At this stage both the xylem and the phloem are entirely primary—that is, of procambial origin. The phloem consists of small groups of sieve tubes with their companion cells and thin-walled conducting parenchyma (Pl. 31, A, B). Phloem fibers are first observed in slightly older tissue. When present, they occur singly or in small groups on the inner face of the endodermis, and in the outer region of the pith. Groups of primary phloem occur not only to the inside and outside of the primary xylem groups but are equally prominent in the interfascicular region where they may be seen at varying distances on both sides of the well-developed interfascicular cambium (Pl. 29, A). The outer phloem groups are small and close to the cambium; the inner are larger and more distant.

Since the structure and development of the phloem are the principal objects of this study, the cells of the xylem are considered only briefly. The vessels are porous, and of the type usually found in herbaceous angiosperms. The vertical extent of the individual cell is about two to three times its diameter. The end walls are usually somewhat oblique (Pl. 33, E), but sometimes nearly transverse (Pl. 32, B). As is usual in vessels, the walls are heavily pitted, the pits being arranged in transverse series (Pl. 33, E). Typical tracheids and wood parenchyma cells are found scattered among the larger vessels. In places these parenchyma cells are arranged in radial rows, forming narrow bands one or two cells wide. These are the innermost cells of the first-formed medullary rays, those extending from the pith itself, rays which may be called "primary medullary rays." In stained sections these cells are distinguished not only by their arrangement, regular size, and shorter tangential diameter but also by their darker stain. The protoxylem elements have already been considered and will be described in detail in the study of the ontogeny.

Just as the vessel is the most important element of the xylem, so is the sieve tube the principal and most interesting element of the phloem. In the potato the tubes have a cylindrical shape, with end walls strictly transverse. A single sieve plate occupies nearly the entire transverse wall, and no plates are found in the radial and tangential walls. The plate appears to be perforated by a large number of circular pores, as is seen clearly in Plate 33, B, and in the enlarged view (Pl. 33, D). Sieve fields as such do not exist in this plant; each large pore of the plate represents the sieve field of forms with primitive phloem. There is little variation in the size of the sieve tube in both inner and outer phloem. The length of the individual sieve-tube segment is, on the average, 138 μ; the diameter of the lumen varies from 17 to 32 μ. Whenever branching of the bundle and anastomosis occurs, the size of the sieve tube varies much more (Pl. 34, A, B). At such places the elements are short and comparatively broad (Pl. 35, A, B). When the sieve-tube mother cells undergo division, the larger of the two cells formed becomes the sieve tube proper; the smaller one, retaining its nucleus, becomes the companion cell. The number of companion cells formed by a single sieve-tube mother cell varies, but as a rule it is not more than one. Sometimes the mother cells do not undergo division, and thus there may occur a series of sieve tubes without companion cells. Besides sieve tubes and companion cells, we find conducting parenchyma in the phloem. These cells are not always distinguishable from the tubes in cross section, since they have about the same size and the same delicate walls. However, in radial section, they are seen to be elongated, rectangular cells, with end walls bearing simple pits very unlike the multiperforate end walls of the sieve tubes (Pl. 32, B).

The phloem fibers are long and awl-shaped, with much thickened secondary walls which later become lignified. A small lumen is usually present in these cells, but pits are wanting. The diameter of the cells varies greatly, fluctuating within the limits of 19 and 40 μ (Pl. 46, A, B).

The cells of the cambium (Pl. 32, D) are of the general shape and proportions of tracheids; the ends are pointed, the terminal walls following an oblique tangential course. In radial section the sloping character of the end walls is not apparent. In cross section (Pl. 30, A) the cambium cells are rectangular, with the greater diameter in the tangential direction. Secondary medullary rays, of course, arise in the cambium from typical cambium cells which undergo a definite number of transverse divisions. These medullary initials persist in the cambium and are seen in tangential section to constitute a part of that tissue (Pl. 32, D).

The remaining cells of the vascular cylinder, the endodermis and the pericycle, resemble closely the cells of these tissues as usually found in herbaceous dicotyledons. The elements of the pericycle are cylindrical parenchymatous cells which vary greatly in size. The endodermis as shown in Plate 29, D, is composed of a single layer of cells which differ

from the adjacent cortical cells in their smaller size, more regular arrangement, and lack of intercellular spaces. The tangential walls usually exceed in length the radial ones, though sometimes the cells are isodiametric. Casparian strips are present, but the lignified area is not always distinctly noticeable. In fixed material the protoplasm is found adhering to these strips, but withdrawn from the tangential walls, and thus forms two slime strings. The cells of the endodermis contain some starch even when all other tissue is empty (Pl. 31, A). This starch content is in some plants the most constant criterion for the identification of the endodermis, since the Casparian strips are not always distinguishable.

Both cortex and pith are made up of irregularly spherical, rather large cells interspersed with small, intercellular spaces. The pith often becomes hollow very early, but in certain varieties it remains almost intact until the plant is mature.

The cells of the collenchyma of the potato plant are prosenchymatous in nature, and the walls are thickened in a highly characteristic manner. The deposition of thickening layers is restricted to the corners of the cell and to certain places in the radial walls (Pl. 29, A, D), giving the lumen of the cell a more or less rounded outline in cross section.

Both the epidermis and the subepidermal layer are made up of brick-shaped cells. Those of the epidermis are more regular and often isodiametric; the tangential walls are slightly arched, the outer ones more than the inner. The outer wall has also a slightly developed cuticle. Here and there in epidermal cells anticlinal walls appear, suggesting late division among these cells. Some of the epidermal cells are specialized to form the guard cells of stomata. Beneath the stomata, chambers occur in the subepidermal cells (Pl. 32, A).

THE LEAF

The leaf, with its petiole, may be considered as a lateral expansion of the stem, and its tissue as derived from and continuous with the latter. As seen in cross section (Pl. 36, C), the vascular tissue of the petiole forms a semicircle, open toward the upper surface. The vascular bundles are surrounded on all sides by cortical tissue which merges into collenchyma just beneath the epidermis. Near the base of the leaf blade the outline of the cross section is semicircular; but toward the base the petiole gradually widens (Pl. 43, C). This widening and consequent flattening causes the amount of cortical tissue to decrease on the convex side—that is, the lower side—and on the flatter side gradually to increase. The vascular tissue consists of distinct groups (Pl. 43, C). The central group is relatively small; the two lateral groups, which are separated from the central one by more or less narrow gaps, are large. Isolated from these groups on each side there occur one or two small strands, which form the outer limit of the semicircle and lie in the petiolar wings. As regards the

detailed arrangement of these tissues, the condition found in the stem prevails (Pl. 36, A). The protoxylem is endarch, its first elements consisting of loosely ringed and spiral cells which are gradually superseded by larger closely ringed or spiral ones. Toward the outside, reticulate and porous vessels are found. Between the vessels, which are arranged in radial series, are uni- and bi-seriate medullary rays and small tracheids. The inner and outer phloem groups are in form and structure similar to those of the stem and will not be treated further. Throughout the petiole a cambium is developed which gives rise to some secondary growth (Pl. 36, A). The elements formed by the cambium are mostly vessels and tracheids, six or eight rows representing the extent of development by this meristem (Pl. 43, C).

The midrib projects both above and below from the surface of the lamina. On the lower side it is prominent and convex in outline. On the upper side it forms an indistinct flat ridge which is only noticeable in cross section of the leaf. As seen in cross section, the vascular tissue of the midrib, like that of the petiole, forms a semicircle, the open side toward the upper surface of the leaf. But here the vascular tissue is not at all, or only partially, broken into individual strands. The cortical tissue is differentiated near the epidermis to form a layer of collenchyma. This layer of collenchyma is rarely more than two cells thick except in the upper projecting ridge, which is composed almost wholly of this type of tissue. A cambium is rarely developed. The cells between the xylem and the outer phloem are parenchymatous and sometimes of rather uniform arrangement, closely resembling those of the cambium. Old material shows that these cells may give rise to some weak secondary growth, appearing most prominent at the base of the leaf, gradually disappearing along the rachis, and not extending to the terminal leaflet.

Both in petiole and midrib large ovoid parenchymatous cells are found between the external phloem groups. These cells are always present, though in varying amounts; their significance could not be determined.

The lateral veins are similar to the midrib in anatomy and morphological structure. The projecting ridges become reduced, and the amount of collenchyma is limited to one layer on the lower surface. The vascular tissue also decreases gradually with the size of the vein. The phloem groups become rarer, and the cells of each fewer. The xylem also becomes reduced till finally the terminal branchlets consist of one or two spiral elements and conducting parenchyma.

The mesophyll of the lamina consists of a palisade layer and spongy parenchyma (Pl. 36, E). The palisade tissue, which lies on the upper side, consists of a single layer of elongated and closely packed cells in uninterrupted contact with one another, except in mature leaves, where they are sometimes separated by narrow intercellular spaces. The palisade layer abuts upon the spongy parenchyma, the cells of which are irregular, loosely arranged, and poorly provided with chlorophyll.

Except for stomatal openings, the epidermis completely covers the leaf and is closely similar to that of the young stem. Stomata are found on both the lower and upper side, but are far more numerous on the lower surface. The stomata as seen in Plate 36, E, are of a simple type. The pores are surrounded by a pair of specialized guard cells which contain numerous chloroplasts; accessory cells are not present. The air chambers are small and are formed by the reduction in the size and by the arrangement of the subepidermal cells.

THE ROOT

A cross section through a small fibrous root (Pl. 37, B) shows a central core of vascular tissue, limited on the outside by an endodermis and cortex. The latter varies in extent with the size and age of the organ, being most prominent in young, small roots, and becoming less conspicuous in old, mature structures. The peripheral cells of the young cortex are covered by a "root epidermis," which, however, in old roots becomes torn and is sloughed off. The traces of vascular tissue supplying the lateral rootlets arise in the pericycle, and are given off directly and without branching or complication. Since the amount of primary structures is very insignificant, and most of the tissue of older roots is secondary in origin, the structure of the root will best be studied in its development, and for that reason will be discussed in detail in the section on ontogeny.

THE STOLONS

The stolons arise exogenously from the underground portion of the stem, which they resemble in structure and arrangement of tissues, except for a reduction in the amount of xylem, and absence of specialized mechanical tissue (Pl. 38, A). There are few xylem elements and these are vessels; tracheids are even less plentiful. Collenchyma is entirely wanting, and the epidermis does not show the specialization found in the aerial portion of the plant. The vascular strands of the stele, which when young are separated by gaps, are later incompletely united by means of an interfascicular cambium, which gives rise to some secondary growth. Fibers are found both in the inner and in the outer phloem, but they appear very late in the ontogeny of the organ. According to Reed (*11*), the endodermis here contains no starch even when the surrounding cortical tissue is crowded with starch grains, and this study confirms his observation, inasmuch as there is far less starch in the endodermis than elsewhere. The reverse is true for the endodermis of the stem. The cells of cortex and pith show no feature of interest, being of the type observed in the stem.

THE TUBER

The potato tuber is morphologically a shortened, thickened stem with scalelike leaves, or leaf scars. The eye in its entirety is a leaf scar with its subtended axil, which contains a suppressed lateral branch with

several axillary buds and undeveloped internodes. The central bud of an eye is most prominent and develops first upon renewal of growth. The spiral of the eyes of the tuber is usually left, though De Vries (7) records right spirals also, the latter type less frequent.

Sections through the mature tuber show several zones of tissue readily distinguishable to the naked eye. These zones are the cortex with its periderm, the vascular ring, and the pith. Of these three areas the vascular tissue is least, the pith most prominent. In the region of the eye the vascular tissue approaches the surface of the tuber and provides vascular connection between the developing buds and the reserve materials stored in the tuber.

The amount of the vascular tissue of the tuber is only slightly greater than that of the stolon; but the individual groups are much separated in the expanded tuber, being only here and there united by interfascicular cambium. The xylem is mostly primary in nature, and only in the region of the larger groups are porous vessels of secondary xylem found. As will be shown in the developmental study of the tuber, the phloem becomes more and more broken up into small strands which are found scattered throughout the cortex and pith. The cortex and the pith differ mainly in the relative amount of cellular density, the cortex being more dense on account of the smaller size of its cells and the larger amount of cell content other than starch.

A periderm 6 to 10 cells in thickness covers the entire tuber. The homogeneity of this layer is broken by small lenticel-like structures which are concerned with the aeration and which have developed below the position of the stomata of the young stolon tip.

THE FLOWER

The flowers are borne on short bractless pedicels which show the histological features characteristic of the stem (Pl. 40, A). The vascular tissue, however, forms a more or less continuous band instead of being arranged in distinct groups (fig. 2, A, a). With the broadening of the pedicel to form the torus of the flower the band of vascular tissue becomes broken through the separation of five vascular strands (fig. 2, B, b), which diverge to occupy a position in the outer cortex—that is, the peripheral region of the cortex (fig. 2, C, b).

When these groups have become distinct—in fact, even a little earlier—almost all of the remaining vascular tissue, c, of the cylinder now more or less reunited (fig. 2, D, c), passes out obliquely to form 10 separate bundles, d, in the inner cortex (fig. 2, E, d). The tissue which does not pass out is in two elongated groups which soon divide (fig. 2, E, e), each giving rise to two small groups of unequal size which, when entirely free, occupy the four quadrants of a circle (fig. 2, E, e; F, e).

Of the 10 inner cortical groups, d, the five which alternate with b begin to expand and divide (fig. 2, F, f). Each one cuts off by radial

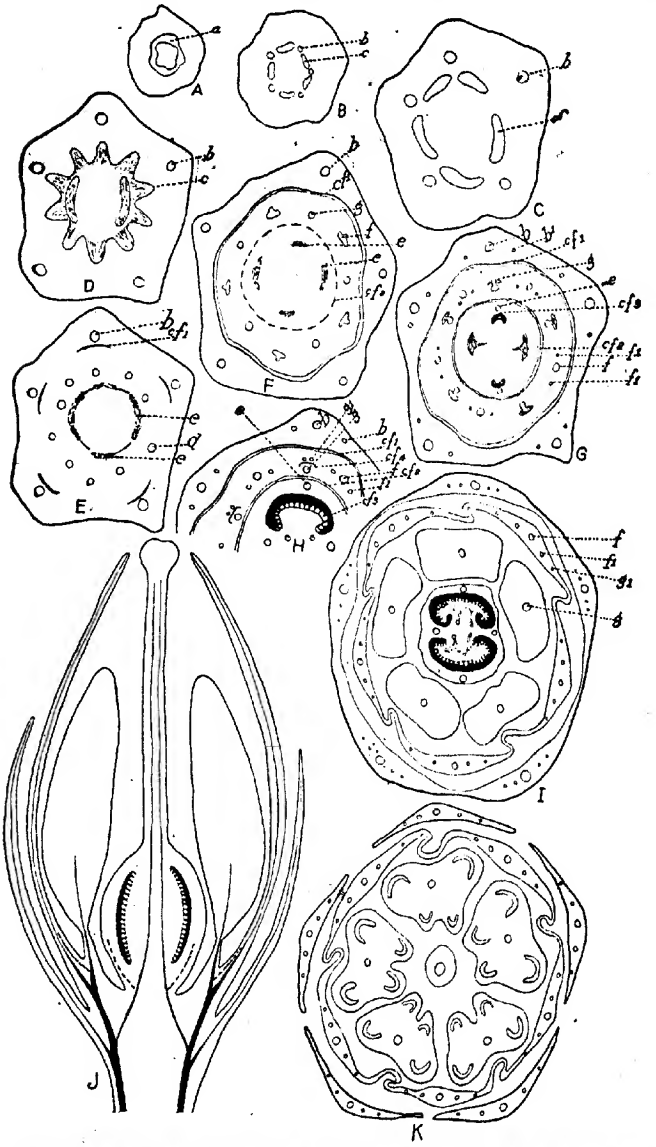

FIG. 2.—*Solanum tuberosum:* Diagrams illustrating the origin and course of the vascular supply of the flower. (See p. 234, 236.)

constriction two small ones, f^1, on opposite sides of a large third one, f, the remainder of the original group (fig. 2. G).

Simultaneously with these divisions a cleavage furrow, cf^1, appears inside each of the five outer cortical groups, b, as shown in figure 2, E. These furrows extend laterally until they meet, cutting off a concentric ring of tissue which represents the first cycle of the flower—that is, the calyx (fig. 2, F).

When the five inner cortical groups, f (fig. 2, G), have completed division, a second set of cleavage furrows, cf^2, originates, cutting off another concentric ring of tissue and leaving in the center a slightly oval area, in which are found the four vascular groups, e.

Simultaneous and progressive changes are now seen in all parts. In the calyx the midrib, b, has given rise to groups of vascular tissue (fig. 2, G, b^1), which in some places are still connected, in other places are already distinct, forming the lateral veins of the calyx lobes. Each of the five bundles (fig. 2, F, g) of the inner cortex which lie opposite the midribs of the calyx lobes cuts off a small amount of vascular tissue to form two distinct bundles (fig. 2, H, g^1). The central portion of the tissue of the torus, which is not differentiated, and which contains the innermost ring of the four bundles, e, now begins to form the ovary proper (fig. 2, G). The first visible change is the appearance of two small openings (fig. 2, G, cf^3), the convex sides of which are directed toward the two smaller of the four vascular groups, e, just mentioned. Serial sections show that these openings become larger; the surrounding tissue becomes the two carpels of the ovary which are united adaxially. The margins of the coherent carpels form more or less evident outgrowths, the placentas, which in turn bear the ovules.

As soon as the cortical groups (fig. 2, H, g^1) have become distinct, a cleavage furrow (fig. 2, H, cf^4) begins to form just below them and gradually advancing tangentially cuts off the second cycle of the flower—that is, the corolla. This cycle is not of equal width throughout, but is constricted and folded at five places to allow for the later expansion of the wheel-shaped corolla (fig. 2, I). The midribs and larger lateral veins of these lobes of the corolla tube are formed by the inner cortical groups f and f^1. The vascular tissue of the smaller veins of the corolla is derived from the five inner cortical groups, g, which were last to divide.

The tissue between ovary and corolla becomes radially cleft to form the five stamens. Each stamen has for its vascular supply a single strand of tissue which is derived from one of the five inner cortical groups, g, opposite the calyx lobes (fig. 2, I, g).

The course of the vascular supply in the flower and the mode of origin and course of the traces supplying the different members of the same is illustrated in the semidiagrammatic sketch (fig. 2, J). Figure K shows a mature though still unopened flower, with its vascular supply in cross section through the anthers just above the distal end of the ovary.

ONTOGENY

THE STEM

A section taken from the tip of a growing potato sprout shows that this region displays no trace of the complicated structure of the older portion of the stem, but is entirely made up of thin-walled cells which are rich in content. Close behind the meristem or true growing region, the uniform cell mass becomes differentiated into distinct layers. The cells, however, retain the abundance of protoplasm, the thinness of the wall, and also the power of division. Farther away from the growing point, the distinctive characters of the tissues become more and more apparent, the organ gradually attaining the differentiation of the mature plant.

The first differentiation in the distal end of a developing sprout consists in the setting off of three distinct regions: the dermatogen, the procambium, and fundamental tissue, including cortex and pith (Pl. 41, A, D). The procambium forms an unbroken hollow cylinder, with small projections into the pith. It is made up of small and elongated thin-walled cells, with abundant protoplasmic content, thereby differing from the surrounding cells of cortex and pith, which are much larger and short cylindrical in vertical section. Almost simultaneous with the setting off of these tissue regions, the first elements of mature vascular tissue appear. They are found most commonly in the small inner projections of the procambium cylinder (Pl. 42, A, D), and are recognized by their slightly larger size and by the secondary thickening of the wall. Longitudinal sections show that these cells are longer than those of the procambium, and further, that the secondary thickenings consist of simple rings located rather distantly from one another. The youngest material examined showed six to eight such elements in one cross section (fig. 3, A). These cells, the first of the protoxylem, then, are the first vascular elements to differentiate from the procambium. It is, however, generally held that the phloem is differentiated at an even earlier period than the xylem, but if such is the case, the phloem cells are not distinguishable from the surrounding procambium cells.

A period during which both growth and differentiation reach their greatest intensity now ensues. The changes consist chiefly of progressive growth and maturation in the procambium cylinder. The latter increases in actual size, both by cell division and cell enlargement. This increase in size of certain of the elements is most marked in the procambium cells at the periphery of the pith, resulting in the setting off of small groups of cells which have not enlarged; the latter cells are the internal phloem group initials (fig. 3, B; Pl. 41, C; 42, B, D). A number of cells near the middle of the procambium cylinder stand out clearly because of their more regular size and orderly arrangement in the form of a tangential band one cell wide. This band, however, is not continuous, but is evident only in those places opposite the procambium

projections (fig. 3, D). More advanced stages in the differentiation of the procambium show these cells to be cambium initials.

While these changes are taking place the number of xylem elements is increased. The later-formed cells appear progressively farther and farther away from the pith, thereby indicating that the development of the protoxylem is proceeding centrifugally (fig. 3, C). Aside from a difference in position, the later-formed protoxylem is characterized by larger size and by a different type of secondary thickening in the form of loose spirals and close rings.

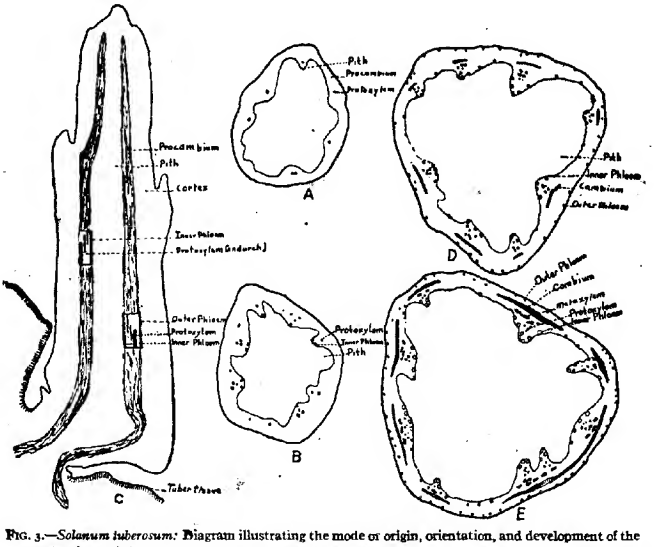

FIG. 3.—*Solanum tuberosum:* Diagram illustrating the mode of origin, orientation, and development of the vascular tissue of the stem. A, B, transverse sections through the distal end of a potato sprout; C, radial section through such a sprout showing vascular connection with mother tuber; D, E, transverse sections farther away from the growing point.

Growth and differentiation become more and more evident; these consist of further changes in the regions already set off, and of specialization in the still undifferentiated part of the procambium. In the external region distinct differentiation also now occurs. The changes, though similar, are less extensive than those noted at an earlier period in the procambial region adjacent to the pith. Thus, a small number of phloem group initials is set off; the number of cells in each of these is also small (fig. 3, D; Pl. 42, B).

Three types of primary vascular tissue are thus early differentiated from the procambium: protoxylem, cambium, and phloem. The phloem groups appear in the innermost and outermost regions of the procambium; the bundles so formed are therefore bicollateral (Pl. 41, B).

Though the protoxylem elements are easily recognized in cross section by the thickness of their secondary walls, the protophloem cells have little to distinguish them from the procambium except their location. In vertical section, however, they do exhibit a few differences, though only under high power. The cells of the inner phloem which lie adjacent to the pith, though having the general proportions of the normal procambium cell, are slightly more elongated, and the content of the cells is richer and more granular. In fixed material the protoplasm has withdrawn from the side walls and has formed a slime string, which in the region of the transverse wall of the cell widens, filling almost the entire lumen. A similar condition is found in the adjacent cell, suggesting a protoplasmic connection between these cells, but actual perforations in the wall are not noticeable as yet. These elements are the first sieve tubes of the phloem, and from their location, it is noticed that they have developed farthest away from the protoxylem elements, differentiating progressively outward. Their development is, then, centrifugal, like that of the protoxylem. A little later, sieve tubes in the outer phloem begin to appear. These are similar in structure in every way to those of the inner phloem, but develop centripetally (fig. 3, D). The sieve tubes of the inner phloem are, then, the first to differentiate, a fact which might have been expected, since here the first visible differentiation of phloem group initials takes place. The number of sieve tubes at such a stage is very small, for rarely are more than one or two parallel rows seen in a section.

A cambium becomes distinct very early in the ontogeny of the stem even before the sieve tubes of the inner phloem are differentiated. The first-formed cambium initials divide rapidly forming two or three tiers of cells in the region of the projecting points of the procambium, long before a tangential connection between all the cells of this layer is established.

The protoxylem elements, maturing at this stage, are still small; they have secondary thickenings in the form of close rings and flat spirals. The secondary thickenings of the first-formed elements, owing to constant elongation of the cells, have become separated and form scattered rings (Pl. 32, D). They are further distinguished from the later-formed elements by their location and smaller size.

In somewhat older stages several changes in progressive differentiation have taken place. Plate 41, B, shows the vascular cylinder at such a stage. The number of protoxylem elements has increased from about 8 to 56, and the phloem groups have more than doubled in number. Though it is not always possible to distinguish the individual groups in their entirety, 76 groups were counted in the inner and 68 in the outer region (fig. 3, E). Still farther away from the distal end is found what is probably the maximum number of phloem groups, 116 in the inner

and 102 in the outer region. The number of protoxylem elements at this stage is about 130.

On taking the whole of a cross-sectional area at this stage, it is noticeable that there has been an unequal differentiation in the procambium cylinder, with greater development and specialization in the region of the procambial projections. These regions have developed to an extent such that under low power six groups of vascular tissue and gaps separating them may be readily differentiated. Gradually, however, differentiation extends also to these interfascicular regions, being initiated by the appearance of a cambium and primary phloem group initials. Plate 31, A, shows the development of such an interfascicular cambium. It does not form simultaneously throughout, but arises in different places, gradually uniting and thereby bridging the gaps between the large groups of vascular strands. Phloem groups are seen outside and inside the cambium. The outer groups are small and closely arranged (Pl. 29, A, C), the inner more or less scattered and usually more distant from the cambium, forming, together with those of the inside of the projections, a more or less symmetrical figure.

In the region of the larger vascular groups large xylem initials are formed (Pl. 29, C; 42, A). The thin delicate primary wall of these cells soon becomes strengthened by secondary thickenings in the form of scalariform and reticulate bands. In vertical section these cells are cylindrical with somewhat sloping end walls. The type of secondary thickening, unlike that found in the protoxylem elements, is such that further elongation of the cell is impossible. This fact, together with difference in size and location, serves to distinguish between protoxylem and these later-formed, larger cells of the primary xylem which are known as metaxylem.

The nature and amount of differentiation which we have thus far followed in the young potato sprout relate to the procambium, which, as we have seen, gives rise to all the primary vascular tissue of the stele. Simultaneous changes in the other meristematic tissues consist chiefly of cell enlargement without marked differentiation. In the cortical region the peripheral tissue undergoes qualitative but not quantitative changes. The two outermost layers are of cells, rather regularly arranged, the elements themselves being more or less rectangular and vertically elongated. Within these we have two or three layers of cells, polyhedral in cross section, and tracheid-like in tangential view. The walls of these cells, however, are thin and of cellulose. With the appearance of the potato sprout above the surface of the soil, these prosenchymatous cells of the cortex develop secondary thickenings in a characteristic manner. Wall thickenings occur in the four corners, sometimes also along the radial walls (Pl. 29, A, D). This specialized tissue of the cortex, the collenchyma, serves as the supporting tissue of delicate, growing organs.

Differentiation of the vascular cylinder continues. The first formed metaxylem elements are now mature; new ones appear continuously.

These are arranged in no constant or typical manner, though often the larger cells form radial rows (Pl. 42, A).

The inner phloem groups meanwhile increase in size and in number of elements. Sieve plates are readily seen in both longitudinal and cross section. The outer phloem groups also become larger and more distinct. In many places they are separated by large, ovoid cells. The interfascicular cambium is almost completed between the six first-matured groups of vascular tissue, and is in places two or three rows wide (Pl. 29, D).

An endodermis has so far not been distinguishable from the cortex, but now becomes fairly distinct. The cells making up this layer are small, usually more regular in shape, and contain starch even when the latter is not present in other tissues. (Pl. 29, C; 31, A.)

The phloem fibers are the last to appear. The first of these are differentiated simultaneously in the inner and outer phloem. At first they are seen singly; later they may increase in number forming groups. Their walls are usually heavily thickened, but do not become lignified until later. In the inner phloem the fibers usually appear in groups either scattered among the peripheral pith cells or forming the inner limit of the phloem groups (Pl. 33, C). In the outer region the fibers usually appear in groups, either scattered among the peripheral pith cells or abutting on the inner phloem groups (Pl. 33, A). In the outer region the fibers form a single broken layer next to the endodermis; they also occur occasionally in groups.

Not all of the procambium cylinder differentiates into conductive elements and fibers. Some of the cells of the outer region enlarge without specialization, forming the parenchyma between the outer phloem groups, the pericyclic region of the stele. In the inner region a similar change takes place. The cells between and around the protoxylem elements and the innermost phloem groups, though differing from the pith cells in being smaller, remain parenchymatous and unspecialized. They form, together with the protoxylem, a band of tissue limiting the pith on the outside and the vascular cylinder on the inside, a band which may be called the "perimedullary zone," or "*Markkrone*" (Pl. 29, C; 30, B). Sometimes typical pith cells separate groups of inner phloem from this region. These isolated phloem groups then appear as though they do not belong to the stele (Pl. 29, A), and, hence, have been called "pith bundles."

Above is in brief the early ontogeny of the tissues and elements of the sprout of the potato plant. In comparison it is of interest to trace the development of a growing tip of a mature stem, and to note differences in order of appearance.

In such an older stem there is already very near the growing point a fairly well differentiated vascular ring. The inner phloem groups are distinct and numerous; the outer groups are still undifferentiated procambium. The protoxylem elements are found singly and scattered;

they are small and few in number. Here differentiation of the phloem seems to be in advance of that of the xylem, or at least it has kept pace with the latter, whereas in the growing tip of a sprout no phloem is found when the first protoxylem elements have become evident. Even at these early stages collenchyma is present. This tissue is obviously here as a supporting structure, since in the underground sprout such tissue is not found. An endodermis has also become visible about this time. The cells are recognized by their regularity, smaller size, and starch content; Casparian strips are not developed.

The other tissues develop in the same sequence as that described in detail for the sprout.

THE LEAF

The petiole and leaf blade may be considered as morphologically a lateral expansion of the stem, and their tissues as continuous with and derived from the latter. A good discussion of the gross morphology of these organs is found in De Vries (*8*). The substance of this is given here, and is followed by the writer's observation on the structure and development of the internal parts.

The leaf primordia appear on the vegetative cone as small protuberances which soon push farther out and curve slightly, the adaxial surface becoming slightly concave. Continuing in this increase and curve, the primordia soon bend over the growing tip, their form thus constantly changing. This cone-shaped growing point, made up of the primordia, bulges on two sides, and soon these swellings can be recognized as the future blades of terminal leaflets. In a very early stage the terminal leaflet consists of a short petiole, a heavy midrib, and blade halves folded together adaxially. For a long time the terminal leaflet is far in advance of the other organs in its development. The latter differentiate only gradually, keeping pace with the elongation of the stem, and thereby providing space for the primordia of the lateral leaflets. The difference in rate of development is so great that the terminal leaflet is already 1 cm. in length and has become green long before the rest of the leaflets appear. The development of the lateral leaflets takes place in basipetal succession; consequently the uppermost leaflets are already green and well grown when the lowermost are still primordia. These lateral leaflets appear as protuberances on the petiole in the same manner as the primordium of the leaf itself appears on the vegetative cone. These are at first quite small, but soon elongate and pass through the same stages as do the halves of the terminal leaf blades.

The first leaf hairs appear very early, developing acropetally on the convex surface of the primordium; later they are formed also on the inner surface in the same order. The hairs appear in two longitudinal rows on the veins, but none are found on the lamina itself. At first only glandular hairs are formed, but soon simple stiff hairs also develop. Both types increase rapidly in number and are mature long before the

internal differentiation of the leaf is complete. When the leaf is still small, the hairs are very numerous and the epidermis over the veins is covered with a dense mat of them; with the elongation of the individual organs the thickness of this mat decreases, since between the already existing hairs no new ones are formed. On the mature leaf the hairs are widely scattered.

Tissue differentiation in petiole, midrib, and veins follows the same general order as described for the stem, but the location and the relative amount and size of the elements is somewhat different. Sections through the middle of a leaf primordium show a small crescent-shaped mass of procambial tissue surrounded by large cells of the fundamental meristem. At first only slight differentiation is noticeable; in about the middle of the procambium a very few protoxylem elements appear. A little farther back from the growing point specialization in the peripheral procambium is going on; the cells are increasing in number and their nuclei are large. Those near the protoxylem at the same time expand and become arranged more or less in tangential rows.

Near the base of a leaf primordium a well-developed procambium area with about eight protoxylem elements is found. At the periphery of the procambium, which has now become semicircular in section, phloem initials appear, forming a band of smaller cells. The first differentiation of the phloem into groups is noticeable in the internal phloem (on the upper side of the procambium). These groups are few and large, and make up a large proportion of the vascular tissue. Here and there the outer phloem (that toward the lower side of the leaf) which had appeared from the procambium as a nearly continuous band, is separated into very small groups. There is, however, considerable variation in the condition found in different leaves of the same size. Often the procambium, though specialized at the periphery, has not as yet formed phloem group initials, whereas in other leaves of the same size almost complete differentiation of the phloem groups has occurred.

In sections through older leaf primordia an increase in the number of protoxylem elements occurs, and the first metaxylem initials, which can be readily distinguished by their large size, have also begun to appear. The cells of that portion of the procambium which has not yet become specialized to form either phloem or xylem, increase in number and size. Those which lie along the convex side of the vascular semicircle have a somewhat regular arrangement thus foreshadowing the appearance of a cambium. Between the outer phloem initials large oval cells become noticeable (Pl. 36, A). Their appearance and location is so characteristic and constant that they would seem to deserve greater attention. Serial sections, however, show nothing suggesting function or structure different from that of the cortical cells. Sections through more advanced stages show both outer and inner phloem arranged in groups which are now quite distinct, and only in the region of the ends

of the crescent do these groups unite, thereby establishing a vascular connection between inner and outer phloem (Pl. 36, A, C). A cambium is developed throughout the crescent of vascular tissue, being more prominent in the region where the procambium has given rise to the largest amount of vascular tissue and less in those regions where specialization is very slight. This cambium later gives rise to some secondary growth (Pl. 44, D).

With the appearance of the first distinct phloem groups, differentiation in the leaf blade begins. Up to this time the tissue of the leaf blade is homogeneous in structure, being made up of brick-shaped cells (Pl. 36, B). An epidermis soon becomes distinguishable and stomata develop. The first cells of the blade to change are those just beneath the upper epidermis. These cells elongate to twice their former length and remain closely packed, forming the palisade initials (Pl. 36, D); in the lower tissue intercellular spaces begin to form, but the size and shape of the cells remain nearly the same. The leaf blade is fully developed, and the cells have completely matured before differentiation in the veins has ceased. The mature leaf blade (Pl. 36, E) has a well-developed epidermis, a palisade tissue one cell deep on the upper side, and three to five rows of spongy parenchyma the cells of which are separated by numerous and large intercellular spaces.

THE ROOT

In plants grown from tubers all of the roots are fibrous in nature, and arise endogenously from the nodal pericycle of the subterranean part of the stem (Pl. 37, A). Transverse sections through this region in a young sprout developing beneath the ground show cells of the pericycle opposite the protoxylem groups richer in content and undergoing division. The cells first elongate radially, and then divide tangentially. The meristematic masses so formed are the root primordia; the central cells of this tissue become the vascular tissue of the young rootlet. The endodermis just opposite the root increases in extent, pushing out as a lobe ahead of the developing rootlet. The latter pushes its way mechanically through the cortex, and is aided by the dissolving action of enzyms which are probably secreted by the cells of the endodermis. Just before the epidermis of the stem is broken the cells of the endodermis cease division and are ruptured, giving way to the rootlet, which then penetrates to the surface and develops independently in the soil.

Transverse sections near the tip of a rootlet which has just broken through the cortex show several distinct zones of tissue. The innermost region, which occupies only a small area, is a solid strand of primary vascular tissue separated from the thick cortex by an endodermis. The epidermis at this stage is specialized, in that many of its cells are elongated to form the root hairs.

The vascular tissue is arranged radially, as is usual in roots, the xylem and phloem in separate strands. These strands alternate with one

another, and the xylem abuts directly on the endodermis. The number of these varies; lateral roots are usually, perhaps always, diarch (Pl. 37, C); others may possess more than these. The maturing of the vascular elements, however, has proceeded in a different direction than was observed in the stem. The first protoxylem elements to mature are those farthest away from the center; the development then is centripetal. The later-formed protoxylem elements of the two groups approach each other more and more closely with the increasing age of the region; those last formed meet in the center. Sometimes, however, a few cells in the center do not become vascular tissue, but remain parenchymatous, forming a pith.

The protoxylem forms two small groups of tissue, somewhat oval in shape, which are separated from the diarch xylem mass by a parenchymatous sheath. Very early a cambium appears; this forms a complete cylinder lying outside the xylem and inside the phloem. Cells are produced by this cambium more rapidly in the latter region and the cylinder soon becomes symmetrical. This secondary tissue is collateral, whereas the primary is radial.

With the continuance of secondary growth, the amount of vascular tissue relative to that of the cortex increases rapidly. Instead of the small vascular cylinder and the huge cortex of the very young root (Pl. 37, C), in the old root there is a large amount of vascular tissue with a comparatively narrow cortex (Pl. 37, D). Most of the tissue of the root is, then, secondary in origin.

The secondary wood of the root consists largely of vessels and wood parenchyma. The vessels are porous and either very large or very small. The large ones are arranged more or less in radial rows; the spaces between them are chiefly filled by the smaller type of vessel. The wood parenchyma, although somewhat scattered, is usually found around the large vessels—that is, it is vasicentric. Occasionally the lumen of one of the large vessels is blocked by bladder-shaped intrusions derived from the membranes of the pits between the vessels and the adjoining parenchyma cells. These vesicles, which are known as tyloses, are very common in many plants, but in the normal potato plant they are not often observed except in the root. The medullary rays of the mature root are few and uniseriate (Pl. 37, D). Typical tracheids are absent, but a few thin-walled fibers are found scattered between the vessels.

Just as most of the xylem is secondary in origin, so is the phloem made up almost entirely of secondary elements. The primary phloem is not recognizable in the mature organ; its cells have become nonfunctional and later are crushed. Most of the phloem cells formed by the cambium are sieve tubes (Pl. 44, C), with their respective companion cells. The sieve tubes are larger than those found in the stem; in general proportion and arrangement, however, they do not deviate from the latter. The cells of the medullary rays of the phloem are slightly broader than those

found in the xylem; their number, of course, is very small. Phloem fibers, as might be expected, are not found in the root.

The cells of the cortex and endodermis are of the type described in detail for the stem. The outer cells of this tissue, since they are not protected by a specialized dermal layer, become somewhat torn and their walls suberized.

THE HYPOCOTYL

Since the change from the exarch condition of the root to the endarch condition of the stem takes place in the region of the hypocotyl, seedlings instead of sprouts grown from tubers had to furnish the material for investigation. The primary vascular tissue of the root develops centripetally, the latest protoxylem elements to mature being found near the center (fig. 4, a).

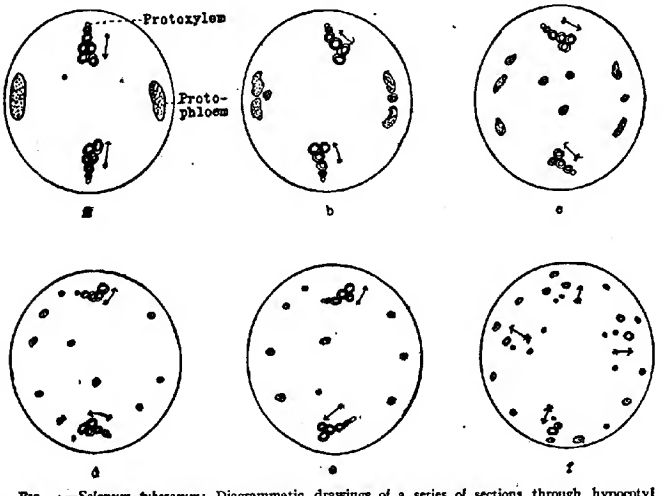

FIG. 4.—*Solanum tuberosum*: Diagrammatic drawings of a series of sections through hypocotyl showing the position of the primary xylem and phloem groups, the changes from exarch to endarch, and the behavior of the phloem.

In the stem the condition is reversed, for here the maturing of the elements takes place centrifugally, and consequently the smallest protoxylem elements are found near the center. In the change from the exarch to the endarch condition it is noticed first, that the two protoxylem groups of a diarch root begin to swing outward, one group following a left, the other a right curve (fig. 4, b). In the region just below the cotyledons the bending has progressed so far that the protoxylem groups instead of forming a radial row, come to lie in a tangential plane. In the region above the cotyledons the change from the exarch to the endarch condition is complete.

Simultaneously with the change in the orientation of the protoxylem in the hypocotyl, go different, though not less important, changes in the phloem. In the root the primary phloem alternates with the xylem, both tissues being arranged in distinct groups. In the stem the arrangement is bicollateral—phloem both inside and outside the xylem. The first noticeable change in the hypocotyl consists in the breaking up of the two phloem groups of the rootlet, with the formation of three to four smaller groups. These phloem strands orient themselves in such a way that two or three of the groups come to lie in the center of the stem between the now separating xylem groups (fig. 4, b, c). The other phloem groups take a position at the periphery of the stele close to the endodermis. At this stage the protoxylem groups have moved through an angle of 90°, and form a tangential row of cells. The outer phloem groups have meanwhile increased further in number so that some of the strands come to lie at the outer face of the protoxylem. The central groups of phloem have also divided and an increase in the size and number of the parenchyma cells in the center of the stele is forcing these groups away from the center and causing them to come to lie close to the protoxylem. The bicollateral condition is thus established (fig. 4, d, e, f).

STOLON AND TUBER

About six weeks after the potato tubers have been planted, branches arise in the axils of scaly leaves of the subterranean part of the stem and grow more or less horizontally outward. These branches, known as stolons, remain simple or fork and sooner or later swell at their tips to form tubers.

The stolons, being modified stems, present a typical stem structure and the tissues go through the same process of development as do those of the latter. In the study of the development of the tuber it is necessary only to note the changes incidental to the enlargement of the stem. In the mature stolon, then, is found a ring of vascular bundles consisting of four or five larger groups with a few smaller ones between them (Pl. 38, A). The xylem is only weakly developed and is made up chiefly of primary elements, among which are a few vessels. The phloem, however, is extensive, and the groups show the typical arrangement in both inner and outer region. The pith and the cortex are made up of large polyhedral cells, many of which are filled with crystal sand (calcium oxalate). A collenchyma and a specialized epidermis are not developed.

The first change in the tip of the stolon consists of extensive cell division in the region of the pith and to a less extent of the cortex, resulting in a swelling of the stolon, which becomes at first oval, later spherical, the change from stolon to tuber being quite abrupt. This excessive cell division in the region of the pith causes the vascular tissue to bend outward; transverse sections at the proximal end of the tuber show the vascular tissue cut obliquely, in places even longitudinally. Potato

tubers of this stage are quite small, not over 5 to 10 mm. thick. The tuber increases both in length and in thickness; the latter interests us chiefly. From this stage on the parenchyma cells of the perimedullary zone, and to some extent those of the cortex also, contribute mostly to the formation of the tuber tissue. The vascular cylinder is forced more and more outward by excessive cell divisions in the regions of the perimedullary zone and pith. However, all of the vascular tissue does not partake in this move. The inner phloem groups become separated with the increase in the tissue of the perimedullary zone and peripheral pith, and gradually split up into numerous strands which traverse these regions of active growth in all directions (Pl. 38, B, C, D).

An examination of the mature potato tuber leads to the conclusion that most of the tuber tissue is derived from the parenchyma of the perimedullary zone of the stele, to a less extent from the parenchyma of the external phloem, the cortex, and the pith. The amount of secondary elements added as a result of the activity of the cambium is insignificant and is limited to a few vessels and some wood parenchyma.

Reed (11), who first followed in detail the development of the potato tuber, maintains that the pith and the perimedullary zone contribute equally to the formation of new tuber tissue; but this does not seem to be the case, since even mature potatoes (in stained sections) show phloem strands in the region near the center—that is, all of the tissue of the tuber except that of the most central part and of the cortex is traversed by phloem strands. The view of De Vries and others that the tuber is formed largely by the activity of the cambium is no longer tenable.

The young tuber has a more or less distinct epidermis with scattered stomata (Pl. 39, A, B). Upon the enlargement of the tuber the epidermis undergoes marked changes. At first anticlinal walls appear in a few of the epidermal cells, probably as a sequence of the tension caused by the expansion of the organs (Pl. 39, C). Later periclinal walls also appear (Pl. 39, D). Simultaneously with the division in the cells of the epidermis division walls also appear in the subepidermal layer. Cell division in this region continues until a layer of tissue is produced which takes over the protective function of the epidermis; this is generally known as periderm or "cork" (Pl. 39, F). The formation and constant regeneration of the periderm are due to the activity of the meristematic cell layer known as the phellogen. In the potato tuber the phellogen consists of a single layer of thin-walled cells which divide tangentially and which constitute the inner row of daughter cells produced by the first division of the cells of the hypodermal layer (Pl. 39, D, E). While most of the periderm arises from the phellogen derived from the hypodermis, the epidermis gives rise to a superficial periderm usually three to four cells in extent. Both layers of periderm tissue, though adjacent to each other, are distinct. The periderm is perforated by a number of lenticel-like structures which arise immediately beneath the stomata, the function of which they assume.

SECONDARY GROWTH

As soon as the intercalary cambium has united the large vascular groups, sometimes even earlier, secondary growth becomes markedly evident. In its beginning the activity of the cambium is noticed only in the region of the large bundles of the stem and is extended only gradually to the interfascicular region, as described in the early part of this paper.

A fully mature stem shows a cylinder of vascular tissue which in the region of the large corner bundles is often more than 2 mm. thick (Pl. 43, B). The xylem in this region contains many large, porous vessels which are more or less regularly arranged. The spaces between the vessels are occupied by tracheids and wood parenchyma. The latter tissue, however, is small in extent, the cells being most commonly found around the large vessels (Pl. 44, A), as is the case in the roots. Medullary rays are very numerous, but the individual ray is narrow, being rarely more than one or two cells wide.

The interfascicular xylem differs from that found in the corners, in that it contains no large vessels, but is made up chiefly of a uniformly arranged mass of tracheids traversed by uniseriate medullary rays (Pl. 44, B). The first-formed xylem elements of this region have smaller lumina and much thicker walls than those later formed. A section through this region resembles strikingly a section of the xylem of a woody stem showing spring and summer growth reversed (Pl. 44, B).

The cambium gives rise on the outside to a comparatively broad ring of phloem which consists mainly of sieve tubes and medullary rays (Pl. 45, A; 47, A). The amount of this secondary phloem varies with the individual and with the place where the section is taken. In the region of the node (Pl. 43, A) the amount usually exceeds that found in the internode (Pl. 43, B); and in a given section the largest amount is found on the face of the large corner bundles. The medullary rays of the phloem widen as they approach the endodermis, and they often bend toward each other in pairs at their tips, inclosing a triangular area of tissue which is made up almost entirely of secondary sieve tubes (Pl. 33, A; 45, B). The primary phloem groups remain functional even after the formation of secondary phloem, and continue active up to the time of maturity of the plant. Their delicate walls, of course, become slightly thickened and occasionally calluses close a sieve plate; the latter, however, occurs only rarely and is probably a pathological condition (Pl. 46, A, B; 47, B).

The cambium gradually diminishes in extent. By the time the vines die, the cambium is in places entirely disposed of—that is, the cells have matured as either phloem or xylem cells.

With an increase in the amount of vascular tissue, the cortex and the pith undergo structural changes. In the region of the large bundles the cells of the cortex parenchyma have become flattened radially owing to

the tangential stretching resulting from an expansion of the vascular tissue which is not accompanied by a corresponding enlargement of the cortex itself. Whenever the expansion is too great to be compensated for by passive stretching of the cells, some of them become meristematic and divide by the formation of anticlinal walls. In the interfascicular region the expansion is much less, and consequently the cells of the cortex retain more or less their original shape. The boundaries of both regions may show transition stages, depending on the amount of secondary growth in the interfascicular region.

The secondary growth which is found in organs other than the stem has received consideration in the chapters on the ontogeny and will not be treated further.

GENERAL DISCUSSION

In the study of the anatomy and the ontogeny of the potato plant as presented above there are given a number of features sufficiently striking to justify reconsideration and discussion.

The protoxylem matures before the protophloem; it is perhaps even first to differentiate. It is usually stated that the phloem precedes the xylem in appearance in the higher vascular plants. The phloem initials appear at first in the inner, then in the outer region, and not in the reverse order, as stated by Weiss (*10*), for the Solanaceae. The maturing of the elements follows the same sequence. The primary phloem groups do not arise by the division of single initial cells, but from groups of small cells set off by the enlargement of surrounding procambial cells. Once set off, these groups enlarge by the formation of new elements.

The bicollateral condition, of course, is characteristic of the Solanaceae, but the inner phloem groups are usually limited to the peripheral region of the pith; sometimes, however, they are found farther away and near the center of the stem, a condition often observed in the potato plant. These innermost phloem groups clearly belong to the stele proper, and do not represent the vestigial remains of a second set of vascular bundles, as is thought by Worsdell (*12*) to be the case in the cucurbits. Their position near the center of the pith is accounted for as follows: A number of the parenchymatous cells of the perimedullary zone divide repeatedly in the radial direction, causing some of the innermost groups to be deflected from their straight course and take a position far within the pith. This type of tissue increase is characteristic of the thickening of the tuber and results in this case in the formation of the extensive sheath of parenchymatous cells which is traversed by numerous small phloem strands.

Of special interest also is the question of the extent of union of the individual phloem groups as they traverse the stem. An examination of Plates 34, A and B, and 35, A and B, shows that these groups branch and anastomose freely. Owing to this, connection is established between the individual groups of both the inner and the outer phloem. A similar

connection occurs between the outer and the inner phloem through the leaf and branch gaps (Pl. 40, B).

Through such a connection the inner and outer phloem become interdependent, just as do the individual groups of either region through branching and anastomosis, as stated above. The physiological importance of the connection of the phloem groups becomes self-evident and must be taken into account when interpreting pathological conditions of the conducting system of the plant.

Secondary growth is quite extensive, and it is necessary to understand the relation of the primary growth to secondary tissues in order to judge correctly their relative importance. It is obvious that there is a great amount of secondary wood formed, but the importance of the secondary phloem seems to have escaped attention; at least it has often been held that secondary phloem does not play a great rôle in the transport of plastic materials. But since the large amount of secondary phloem formed consists chiefly of sieve tubes, it seems self-evident that it is of primary importance in translocation. Before the time of tuberization the movement of plastic materials is localized, most of the organic food being used in the building up of new tissues and for respiration. For the comparatively small downward movement the primary phloem is sufficient, and little or no secondary phloem develops. At the time of flowering, when tuber formation is under way, secondary sieve tubes are formed in large numbers. But while the secondary phloem is formed and takes part in the translocation of plastic materials, the primary groups remain active until the plant is mature. Of course, the delicate walls of the phloem elements become somewhat thickened, but this is a condition to be expected in older structures.

The process of tuber formation has been treated by Reed, whose observations this study confirms and extends. But while Reed believes most of the tuber tissue to be formed by the pith and the perimedullary zone, the writer is led to conclude that the pith does not contribute much to the formation of new tissues, but that it is especially the perimedullary zone which forms most of the tuber. There is further a divergence of opinion in regard to the origin of the periderm. De Vries (*7*, *8*), states that the periderm is formed by the epidermis, whereas Reed (*11*) shows figures to prove that it arises from the hypodermis. A study of the series of photographs (Pl. 39, A–F) shows that, though most of the periderm is formed from the hypodermis, a superficial periderm several cells thick is formed by the epidermis.

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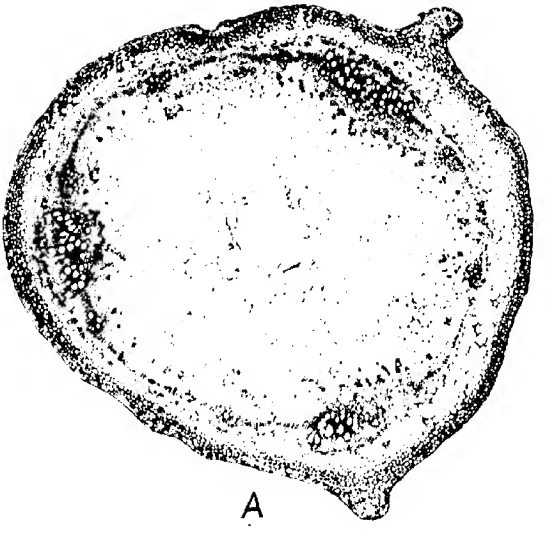
PLATE 27

Origin of leaf and branch traces of the potato:

A.—Transverse section through internode of a partly mature stem. ×13.

B.—Transverse section through a stem immediately below a node, showing the origin of a new trace at *a*. ×13.

(See also Plate 28.)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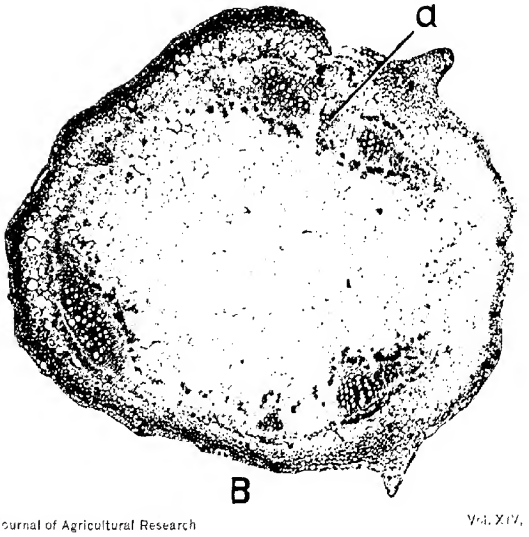
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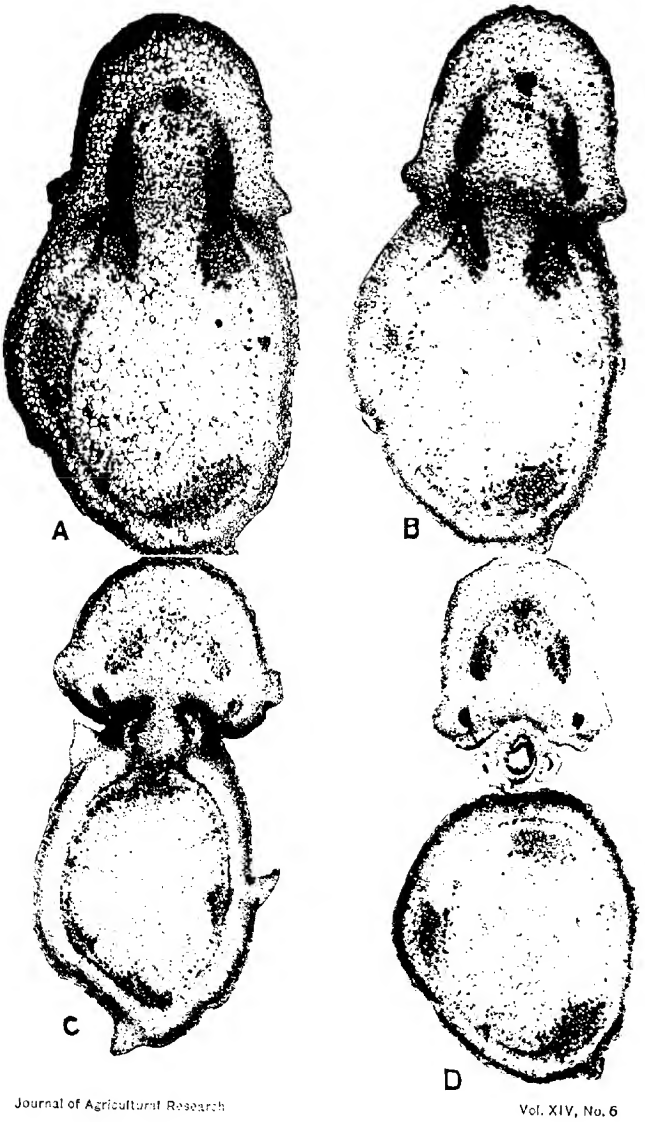
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PLATE 28

Origin of leaf and branch traces of the potato:

A–D.—Transverse sections through successively higher nodal regions, showing the origin of the lateral leaf traces and of the branch trace. ×9.

(See also Plate 27.)

PLATE 29

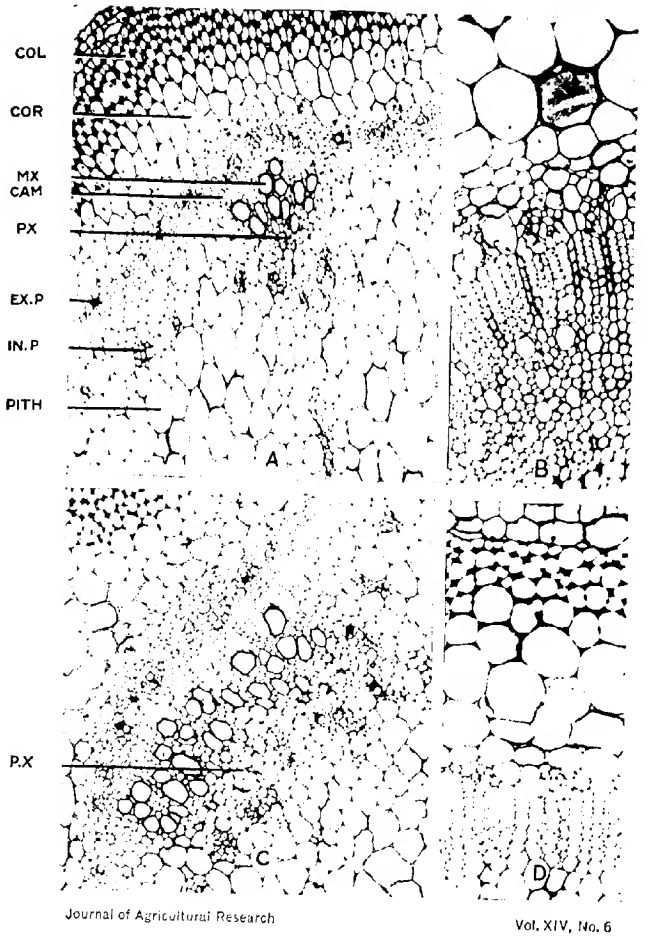
Distribution of tissues (primary) in the potato:

A.—Transverse section of part of the central cylinder and the cortex, showing small stem bundle, extent and position of external and internal phloem, proto- and meta-xylem, collenchyma and cortex, cambium, and pith. ×94.

B.—Transverse section through part of large stem bundle showing fascicular cambium and medullary rays.

C.—Transverse section through a large stem bundle. ×94.

D.—Transverse section through somewhat older stem, showing interfascicular cambium and position of external phloem groups and endodermis.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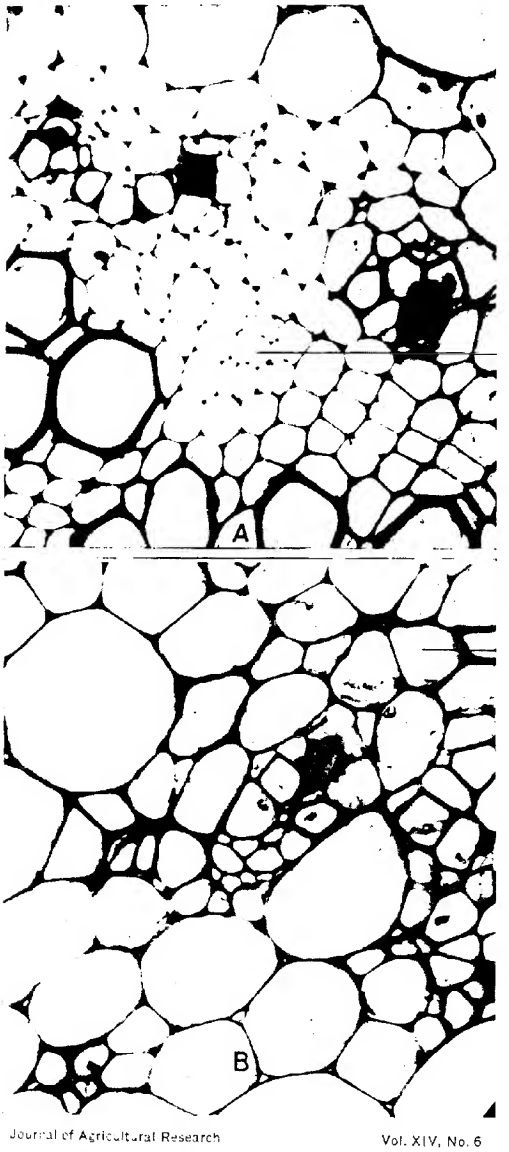

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PLATE 30

Types of primary tissues of the potato:

A.—Transverse section of part of large stem bundle showing sieve tubes and companion cells in outer phloem, type of cambium and medullary ray initials, metaxylem. ×500.

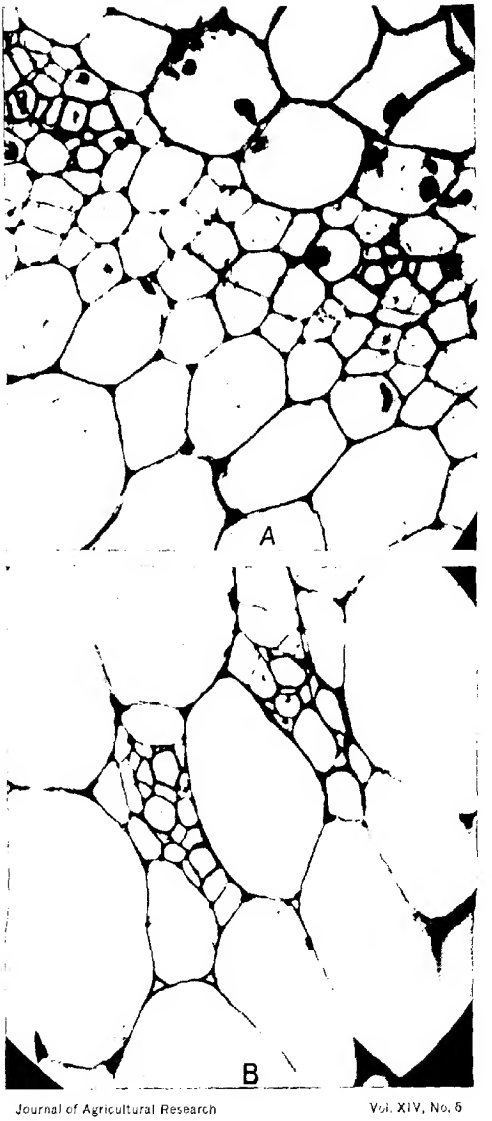
B.—Transverse section through part of the same bundle showing inner phloem, pith, and cells of the perimedullary zone. ×500.

PLATE 31

Types of primary tissues and elements of the potato:

A.—Transverse section through interfascicular region of central cylinder showing cambium, outer phloem groups, and endodermis. ×500.

B.—Transverse section through the same region showing internal phloem groups. ×500.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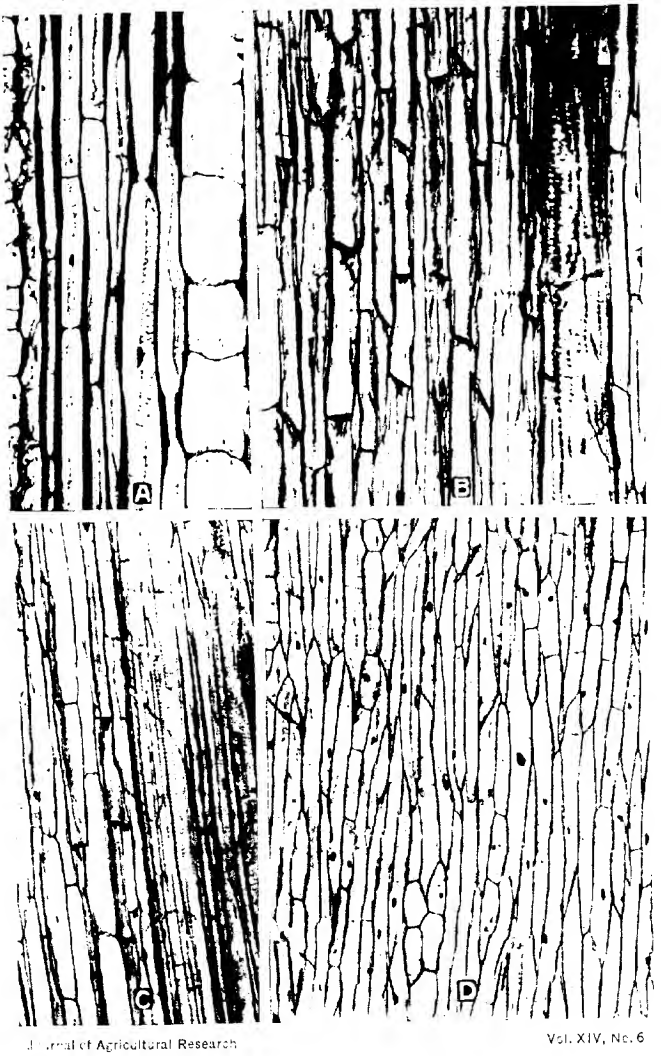
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PLATE 32

Types of elements of the potato:

A.—Radial section of outer cortex, collenchyma, subepidermal layer, and epimeris with stomata. ×109.

B.—Tangential section of part of vascular cylinder of partly mature stolon showing numerous sieve tubes and two porous vessels. ×218.

C.—Tangential section of young stem showing sieve tubes of internal phloem and protoxylem. ×109.

D.—Tangential section of partly mature stem showing cambium and medullary ray initials. ×104.

PLATE 33

Distribution of tissues and type of elements of the potato:

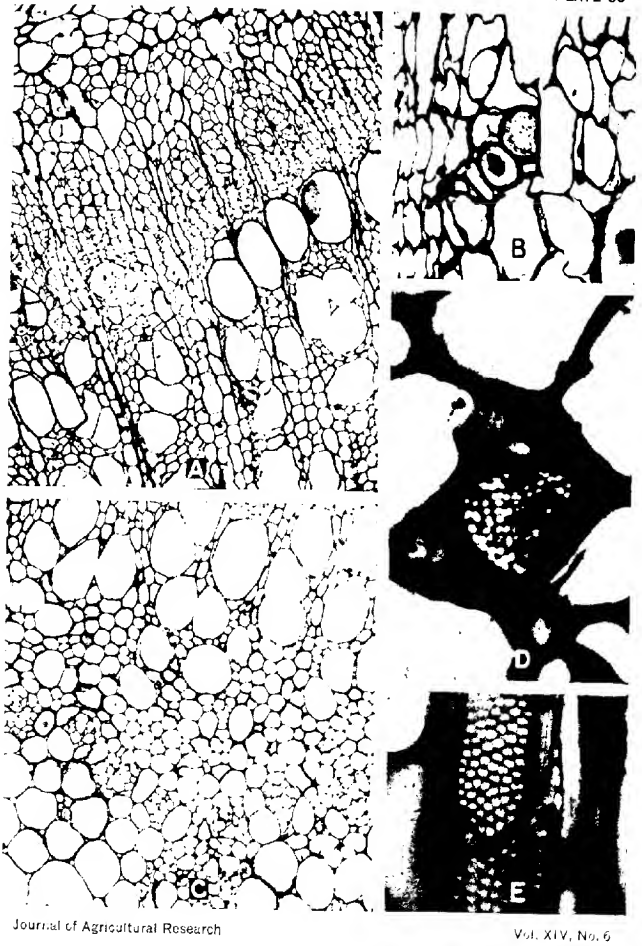
A.—Transverse section of large stem bundle at time of secondary growth showing distribution and type of medullary rays. ×103.

B.—Transverse view of sieve plate of secondary sieve tube. ×405.

C.—Transverse section through a large stem bundle showing type of cells of perimedullary zone and the extent of the latter. ×103.

D.—Transverse view of sieve plate greatly enlarged. ×90.

E.—Radial section through porous vessel, showing type of end wall and extent of pitting. ×292.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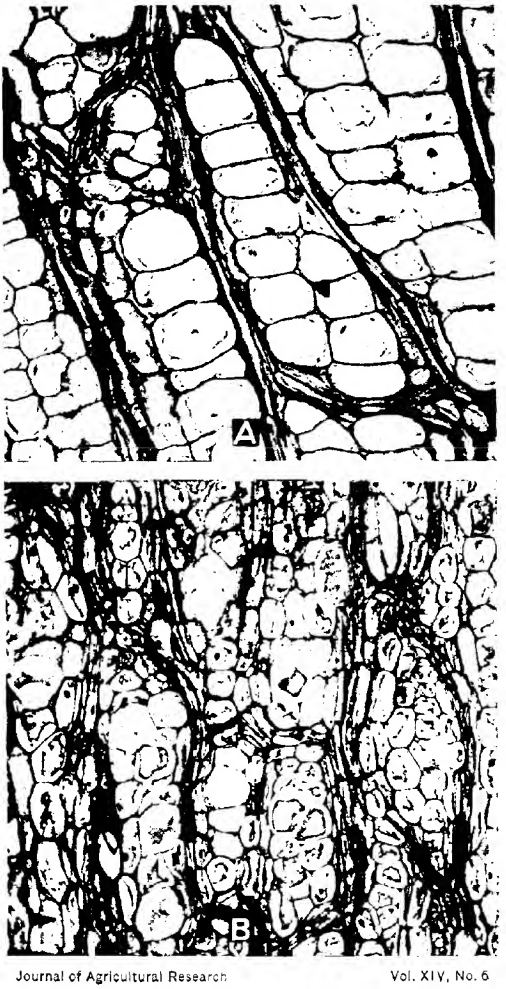
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PLATE 34

Distribution of tissues of the potato:

A.—Tangential section through external phloem, showing branching and anastomosing of phloem groups. ×118.

B.—Tangential section through internal phloem, showing branching and anastomosing of phloem groups. ×112.

PLATE 35

Types and anastomosis of sieve tube of the potato:

A.—Enlarged view of part of Plate 34, B, showing type of sieve tube. ×400.

B.—Enlarged view through another region of the same figure, showing type and anastomosing of sieve tubes. ×400.


A
B

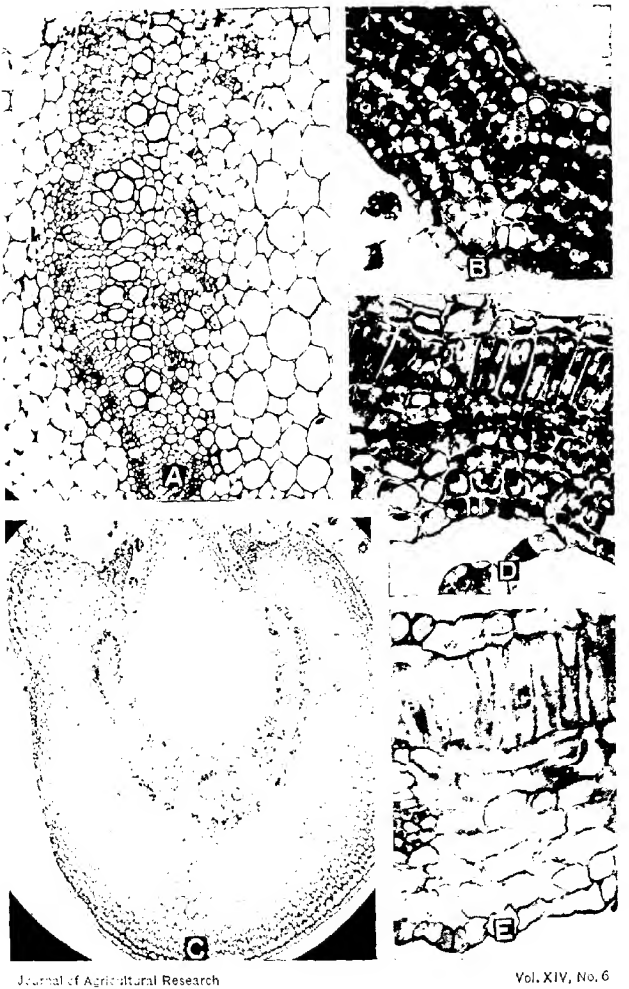
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PLATE 36

Distribution of tissues of the potato:

A.—Transverse section of lateral bundle of mature petiole, showing distribution of external and internal phloem and amount and arrangement of xylem. × 396.

B, D, E.—Cross sections through leaf blade at different stages of development, showing differentiation of palisade layer and spongy parenchyma. × 369, 342, 405, respectively.

C.—Transverse section of petiole, showing arrangement of vascular tissue, amount of cortex, and distribution of collenchyma. × 42.

PLATE 37

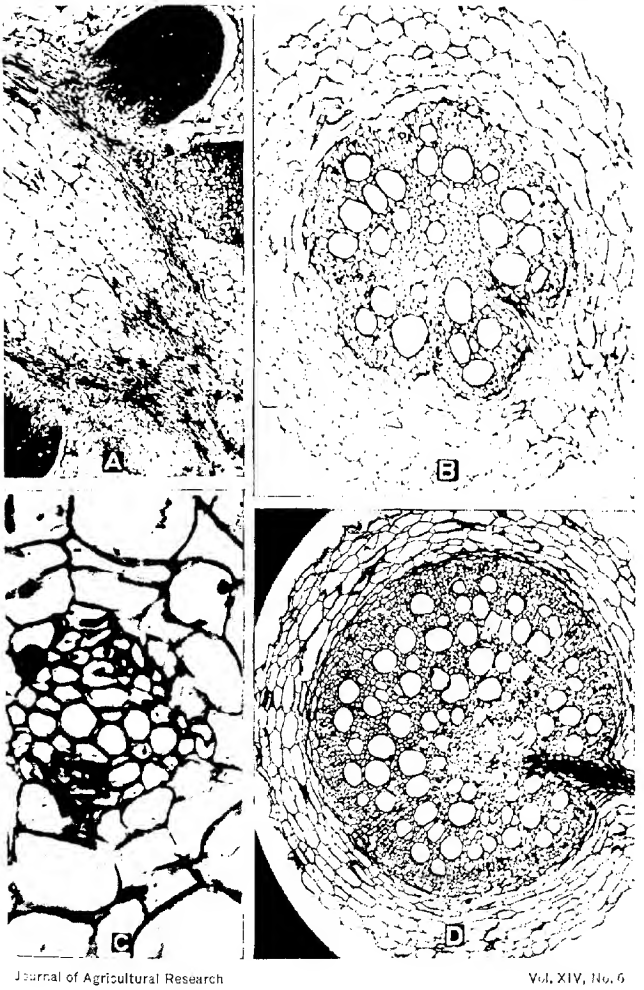
Root of the potato in development:

A.—Radial section through nodal region of underground part of stem, showing origin of roots from the pericycle. × 36.

B.—Transverse section of partly mature root. × 90.

C.—Transverse section of a young diarch rootlet, showing arrangement of protoxylem and protophloem. × 648.

D.—Transverse section of fully mature root. × 54.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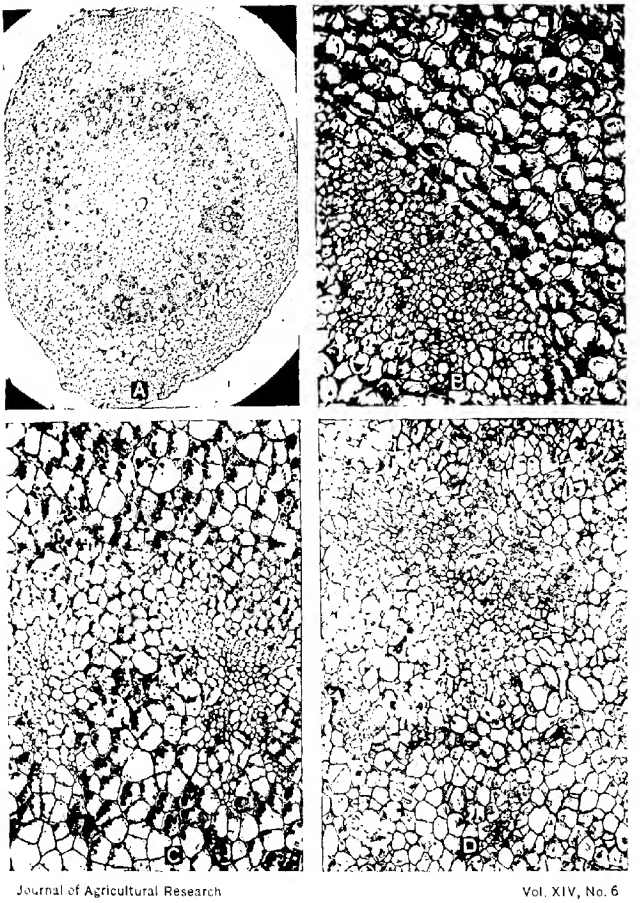
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PLATE 38

Development of the tuber of the potato:

A.—Section of mature stolon, showing general distribution and relative proportions of tissues. × 30.

B.—Transverse section of part of vascular tissue of young tuber and cortex. × 63.

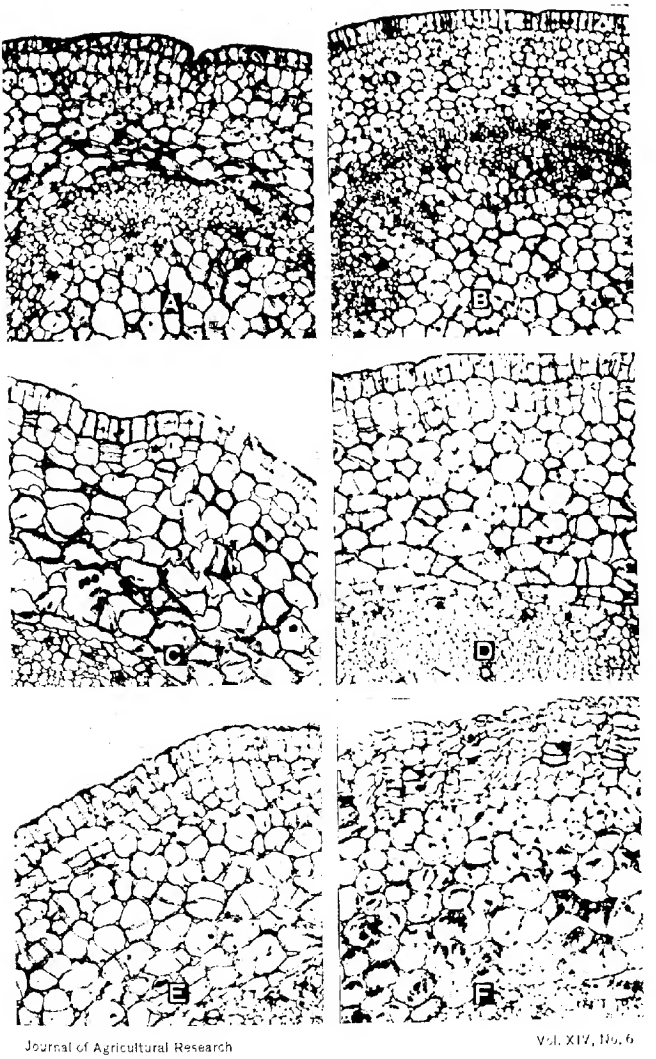
C.—Transverse section of part of vascular tissue of a somewhat older tuber, showing the beginning of extensive cell division in perimedullary zone and parenchyma of outer phloem. × 63.

D.—Transverse section of vascular tissue of partly grown tuber, showing the distribution of the phloem groups after a period of extensive growth in the perimedullary zone. × 63.

PLATE 39

Development of the tuber of the potato:

A–F.—Transverse sections through parts of tubers at successive stages of development, showing origin and development of the periderm. × 99.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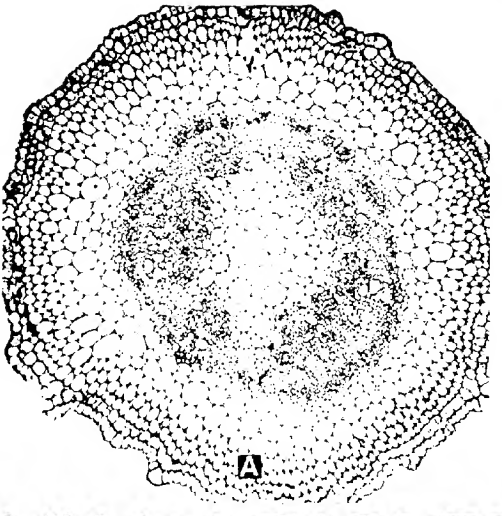
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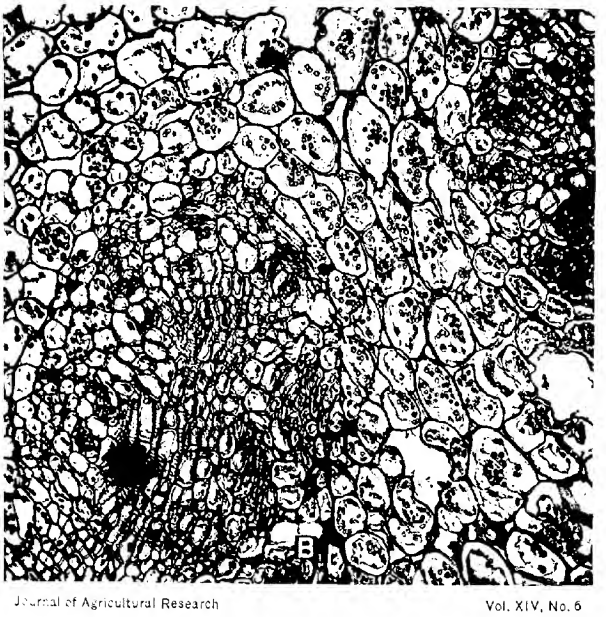
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PLATE 40

Flower pedicel and stem node of the potato:

A.—Transverse section through pedicel of flower. × 46.

B.—Portion of transverse section through node, showing part of wing bundle of petiole above, part of stem bundle below, and leaf gap in center with connection of inner and outer phloem along the side of the stem bundle. × 180.

PLATE 41

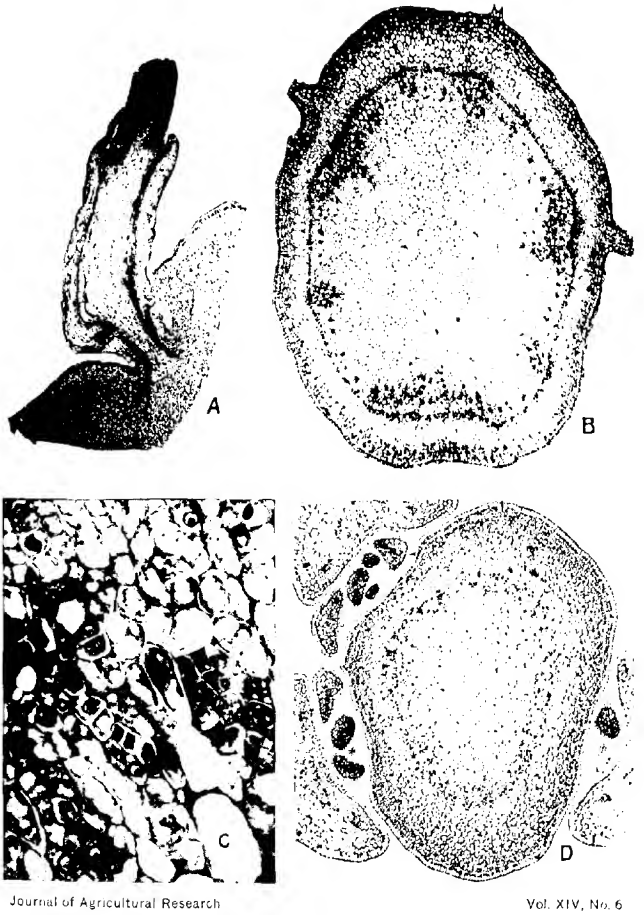
Ontogeny of the potato:

A.—Radial section of potato eye and part of mother tuber showing amount and position of procambium. × 5.4.

B.—Transverse section through tip of potato stem, showing the general distribution of tissues, the amount of vascular tissue, and its arrangement into groups. × 39.

C.—Transverse section through growing region of potato eye, showing the first visible differentiation of internal phloem groups. × 414.

D.—Transverse section through tip of potato eye, showing the arrangement of the various parts and the beginning of vascular differentiation. × 22.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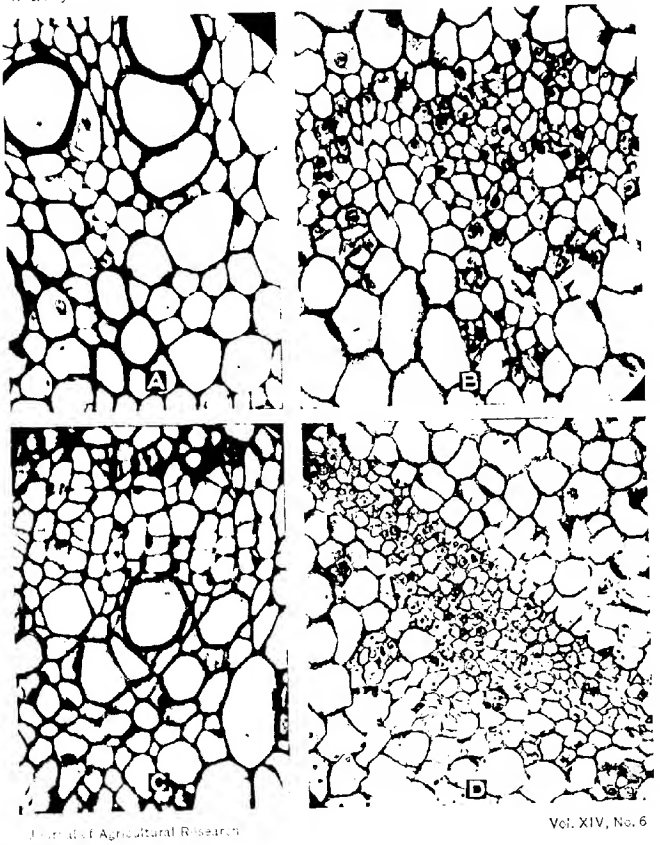
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PLATE 42

Ontogeny of the potato:

A.—Transverse section through potato sprout showing metaxylem and primary medullary rays. × 450.

B.—Transverse section through part of growing region of potato sprout, showing position of the first formed protoxylem and the differentiation of the internal and external phloem groups from the procambium. × 405.

C.—Transverse section through potato sprout, showing beginning of cambium development. × 396.

D.—Transverse section through distal region of potato sprout, showing the first differentiation of internal phloem and protoxylem (X). × 342.

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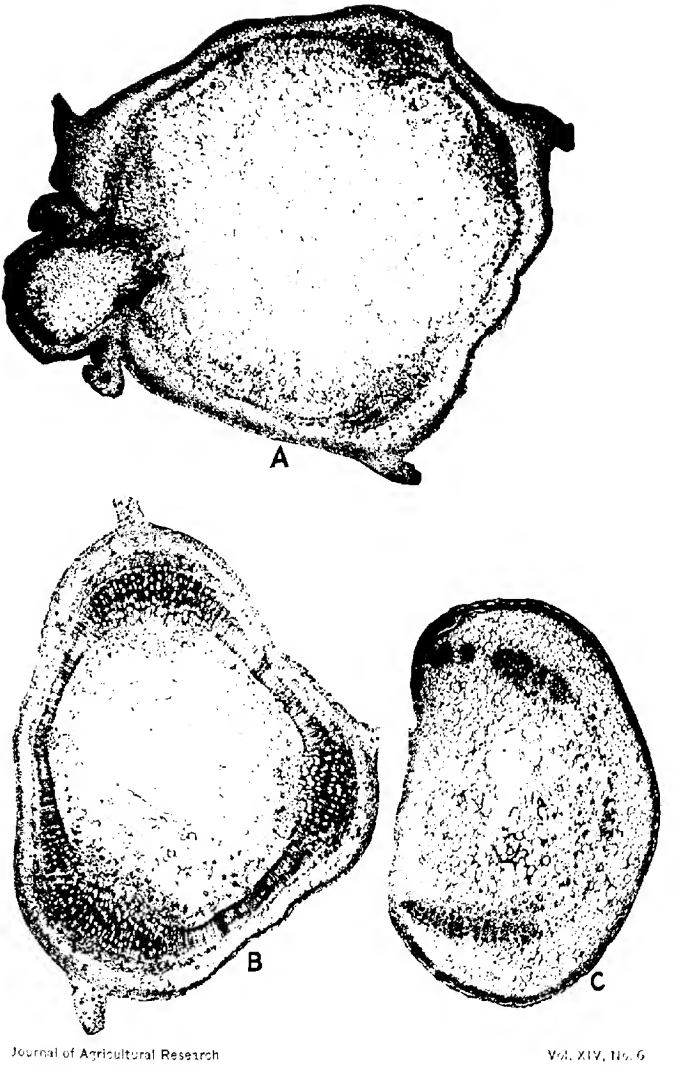
PLATE 43

Secondary growth of the potato:

A.—Transverse section through nodal region of mature stem. × 6.
B.—Transverse section through internode of mature stem. × 9.
C.—Transverse section through mature petiole. × 9.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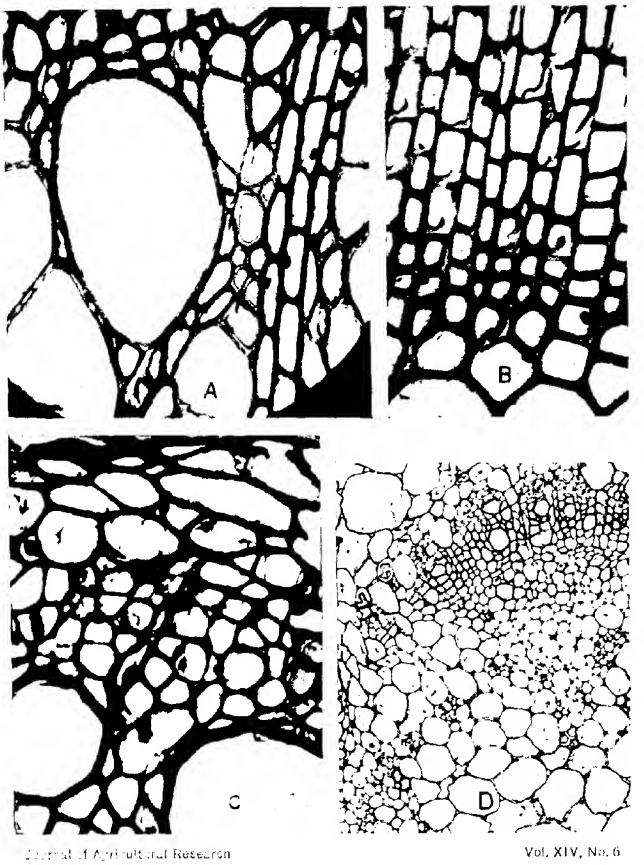
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PLATE 44

Secondary growth of the potato:

A.—Transverse section through part of large stem bundle, showing type of xylem and medullary ray cells. × 234.

B.—Transverse section through interfascicular region of mature stem, showing the types of first and later formed secondary xylem elements. × 234.

C.—Transverse section through part of mature root, showing secondary phloem. × 540.

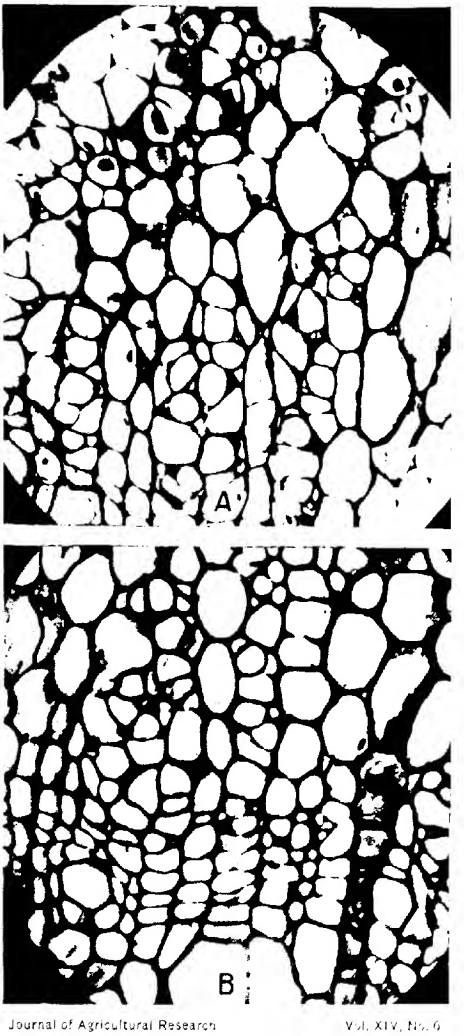
D.—Transverse section through part of mature petiole, showing secondary xylem. × 360.

PLATE 45

Secondary growth of the potato:

A.—Transverse section through phloem of mature stem, showing most of the secondary elements to be sieve tubes and rays. × 400.

B.—Transverse section through another region of mature stem, showing secondary phloem and rays. × 400.


A
B

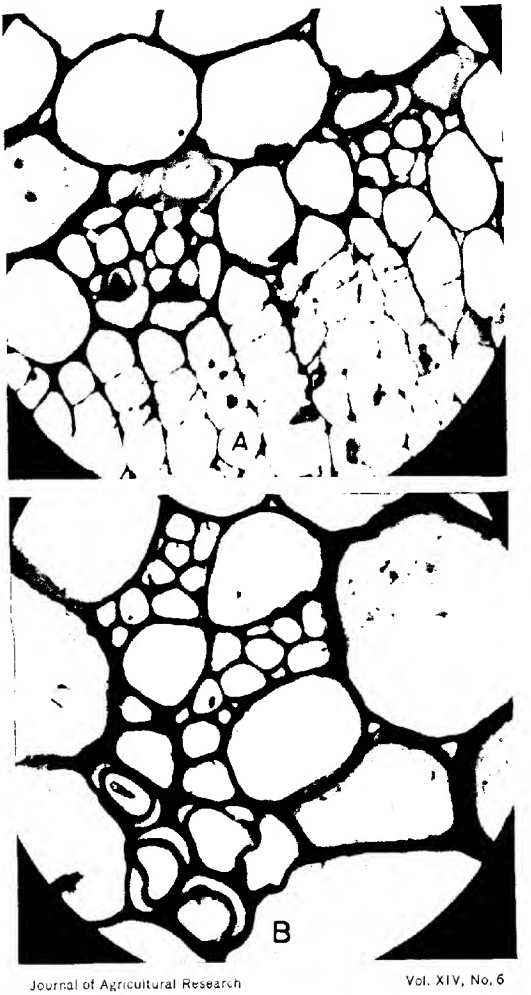

A
B

PLATE 46

Condition of the primary phloem in mature stems of the potato:

A.—Transverse sections through mature stem, showing primary phloem groups in interfascicular region. × 400.

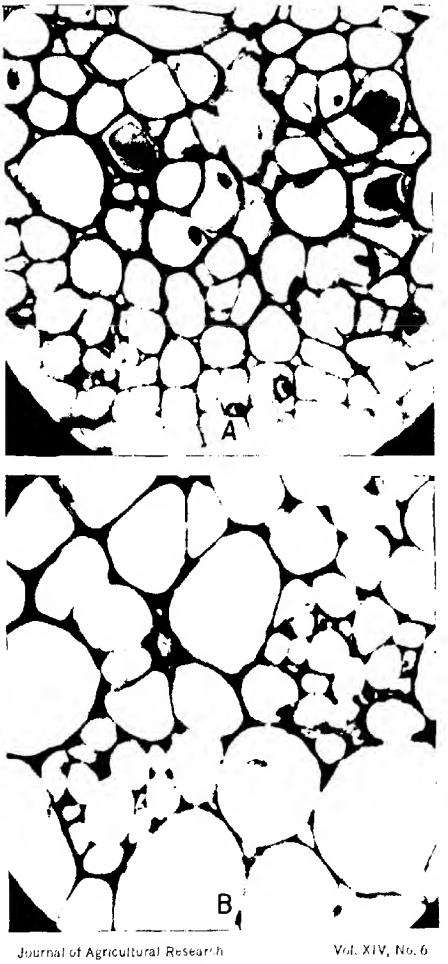
B.—Transverse section through the same region, showing primary internal phloem. × 400.

PLATE 47

Condition of primary phloem in mature stems of the potato:

A.—Transverse section showing that most of the secondary phloem is made up of sieve tubes and ray cells. × 400.

B.—Transverse section through mature stem, showing large internal phloem groups. × 400.


A
B

IMPROVED METHODS OF IMMUNIZATION AGAINST SYMPTOMATIC ANTHRAX (BLACKLEG)

By R. A. KELSER

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INTRODUCTION

During the past several years exceptional interest has been manifested by various investigators in the United States in prophylaxis against symptomatic anthrax. Up to that time the method of immunization practically exclusively employed in this country for the prevention of the disease was that of Kitt's, or a modification of his method.

Since 1897 the Bureau of Animal Industry of the United States Department of Agriculture has prepared and distributed to stock owners throughout the country millions of doses of blackleg vaccine, employing in its preparation the method outlined by Kitt with some modifications. The principle of the method [1] lies in the attenuation of affected muscle tissue from animals that had died of blackleg, and is accomplished by subjecting the same to a temperature of 95 to 96° C. for a period of six hours. The finished product consists of finely powdered muscle tissue containing the attenuated organisms. The results obtained from the use of this vaccine have been very satisfactory, and its use has been a leading factor in the control of the disease in the United States and elsewhere.

At the present time, however, there are, of the more recently introduced products, two which bid fair to surpass in efficacy the various other agents for immunization against blackleg. One of these represents a so-called "germ-free vaccine" or "natural aggressin," and is a sterile filtrate prepared from affected animal tissues. The other is a toxic culture filtrate and is prepared from cultures of the bacillus of symptomatic anthrax produced with special culture media for toxin production and subsequently rendered free from organisms by filtration through bacteria-retaining filters.

Both of these biological products have been found to possess valuable immunizing properties against blackleg, and their methods of preparation are such that the finished products are superior to the powdered vaccine.

[1] For details of this method see NÖRGAARD, V. A. BLACKLEG: ITS NATURE, CAUSE, AND PREVENTION. U. S. Dept. Agr. Bur. Animal Indus. Circ. 31, 23 p. 1900.

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Aug. 5, 1918
Key No. A-40

Numerous tests and experiments have been conducted with both agents, but from the standpoint of production of the two products the efforts of the writer have been concentrated principally on the preparation of the toxic culture filtrate.

GERM-FREE VACCINE OR NATURAL AGGRESSIN

Based on Bail's [1] work on aggressins, Schöbl [2] tried and was able to immunize calves and guinea pigs against blackleg by vaccinating them with sterilized edematous fluid from animals which had died of the disease, the sterilization of the edematous fluid being accomplished through treatment with toluol.

Franklin and Haslam [3] conducted numerous experiments with an immunizing agent based on this same principle and emphasized its value in the prevention of blackleg. The writer has prepared one lot of such a product and has tested with very satisfactory results a number of specimens of similar material received from various outside sources. The following procedure is followed in preparing the product.

Susceptible animals are inoculated intramuscularly with an emulsion prepared from the affected muscle tissue of animals dead of blackleg. The animals usually succumb to the disease in from 36 to 48 hours. The skin is then removed, and the edematous fluid from the affected area and the affected muscle tissue is collected. The tissue is then finely ground and together with the edematous fluid collected is placed in fruit jars and is frozen, an ice-salt mixture being used. After several hours' freezing the jars are removed and inverted over funnels containing thin films of cotton, the funnels all draining into a pan which converges to the center, from which by means of a spout the thawed fluid is discharged into a bottle. Ice is kept packed around the bottle in order to keep the fluid at a low temperature, as the process of thawing requires considerable time even in warm weather. This freezing process is necessary to facilitate filtering. After the dripping from the jars ceases, the "clots" are pressed to extract more of the fluid, but the material thus obtained is kept separate from the other fluid and is filtered last, as it passes through considerably slower and tends to clog the apparatus. The product is filtered twice through Berkefeld filters (first through one of "V" and then through one of "N" porosity), is preserved with chloroform (0.5 per cent), and is ready for testing.

[1] BAIL, Oskar. VERGLEICHENDE UNTERSUCHUNGEN ÜBER MILZBRANDFEINDLICHE EIGENSCHAFTEN IN ORGANISMUS DES HUNDES UND KANINCHENS. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 27, No. 1, p. 10–21. 1900. —— UNTERSUCHUNGEN ÜBER NATÜRLICHE UND KÜNSTLICHE MILZBRANDIMMUNITÄT. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 33, No. 5, p. 343–353. 1903.

[2] SCHÖBL, Otto. WEITERE VERSUCHE ÜBER AGGRESSINIMMUNISIERUNG GEGEN RAUSCHBRAND. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 62, No. 3/4, p. 296–304. 1912.

[3] FRANKLIN, O. M., and HASLAM, T. P. THE STRENGTH AND COMPOSITION OF BLACKLEG VACCINES. *In* Jour. Infect. Diseases, v. 19, no. 3, p. 408–415. 1916.

POTENCY TESTS OF THE GERM-FREE VACCINE OR NATURAL AGGRESSIN

A number of tests have been carried out on calves and guinea pigs to determine the potency of this biological product and the results obtained have been very satisfactory. The samples tested were from various sources, the greater part of them being submitted by commercial houses in connection with applications for licenses to market the product interstate.

The early tests of this product on guinea pigs were not nearly so satisfactory as the tests on calves, owing in a large measure to the character of the virus employed for the subsequent infection of the vaccinated guinea pigs. This test, however, has been much improved through the use of a different type of virus which is described on page 260.

The results of all the tests carried out with this product will not be tabulated, but the following three tests are given as examples of the results uniformly obtained (Tables I–III).

TABLE I.—*Results of subcutaneous inoculation of guinea pigs with germ-free vaccine followed after 14 days by intramuscular inoculation with blackleg virus*

Guinea-pig No.	Amount of vaccine.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	1	0.5	Dead of blackleg in 24 hours.
2	1	.5	Marked swelling but remained alive.
3	2	.5	Dead of blackleg in 30 hours.
4	2	.5	Dead of blackleg in 48 hours.
5	3	.5	Slight swelling, remained alive.
6	3	.5	Do.
7	4	.5	Dead of blackleg in 36 hours.
8	4	.5	Remained alive.
9	Control.	.5	Dead of blackleg in 24 hours.
10	...do....	.5	Dead of blackleg in 32 hours.

[a] The virus employed in this instance was prepared by emulsifying 10 gm. of ground affected muscle tissue from a calf dead of blackleg with 30 cc. of physiological salt solution, and filtering through a thin film of cotton.

TABLE II.—*Results of subcutaneous vaccination of calves followed after 14 days with intramuscular inoculation of blackleg virus*

Calf No.	Amount of vaccine.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	5	10	Remained alive.
2	5	10	Do.
3	Control.	10	Died of blackleg.
4	...do....	10	Do.
5	...do....	10	Do.
6	...do....	10	Do.

[a] The virus consisted of a heavy suspension of emulsified affected muscle tissue.

The use of a relatively large number of controls in the calf inoculation tests was due to the fact that these experiments were made at times when calves were to be infected for use in the preparation of the regular blackleg vaccine for distribution by the Bureau of Animal Industry. At such times four to six calves are usually infected; therefore in order to add to the value of the test four such animals as controls were employed.

TABLE III.—*Results of subcutaneous vaccination of calves followed after 5½ months with intramuscular injections of blackleg virus*

Calf No.	Amount of vaccine.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	5	10	Remained alive.
2	5	10	Do.
3	Control.	10	Died of blackleg.
4	do.	10	Do.
5	do.	10	Do.
6	do.	10	Do.

[a] Virus employed of same character as described in Table II.

PREPARATION OF THE TOXIC CULTURE FILTRATE

Leclainche and Vallée[1] and others have demonstrated that the bacillus of symptomatic anthrax when grown under favorable conditions produces a true toxin. It has also been demonstrated that animals susceptible to blackleg could be effectively immunized against the disease by injecting them with small amounts of such toxin-containing filtrates. Considerable attention has been paid to this method in Japan by Nita,[2] and in this country Eichhorn [3] has recently called attention to it.

It was at first thought that this toxin was of a very stable nature and not materially affected by such influences as air, light, moderate degrees of heat, drying, etc. Subsequent investigations, however, have shown that such is not the case, but, on the contrary, under various conditions, it is more or less unstable. Therefore, in the production of this toxin this fact must be borne in mind, and precautions taken throughout the process to guard against influences likely to affect the material.

Numerous types of media have been prepared and tested for the production of this toxin, and considerable difference has been found in the potency obtained with the various kinds of media. A description will be given only of the type of medium the writer considers most efficient for toxin production, but the following are other medias tried: Dextrose-veal bouillon plus cubes of beef, dextrose-veal bouillon

[1] LECLAINCHE, E., and VALLÉE, H. RECHERCHES ÉXPÉRIMENTALES SUR LE CHARBON SYMPTOMATIQUE. *In* Ann. Inst. Pasteur, année 14, no. 4, p. 202–223. 1900.

[2] NITA. UNPUBLISHED RESULTS.

[3] EICHHORN, Adolph. STUDIES IN BLACKLEG IMMUNIZATION WITH SPECIAL REFERENCE TO BLACKLEG FILTRATE. *In* Jour. Amer. Vet. Med. Assoc., v. 52 (n. s., v. 5), no. 6, p. 653–669. 1918. Discussion, p. 663–669.

plus sterile bovine serum, dextrose-liver bouillon, dextrose-liver bouillon plus cubes of beef, dextrose-veal bouillon plus calcium lactate, dextrose-liver bouillon plus calcium lactate.

The medium with which best results were obtained is a modification of Martin's peptone bouillon, and is prepared as follows:

Fresh pig stomachs with their contents are obtained, and after trimming away the fat, are opened and the contents expelled. They are then lightly rinsed in water, care being taken not to wash away the gastric mucosa. The material is then cut in pieces of suitable size for a meat-chopping machine and finely ground. To every 200 gm. of this finely ground stomach tissue are added 1 liter of water at 50° C. and 20 cc. of hydrochloric acid, C. P. This mixture should be made in glass flasks, and from here on up to the time the material is neutralized it should not come in contact with metal. The mixture is placed in an incubator maintained at approximately 50° and allowed to remain there for 20 to 24 hours. It is then filtered through several thicknesses of cheesecloth, heated to 80° to stop peptonization, allowed to cool down to 70°, and neutralized to litmus. Flocculation occurs at this point, and the material is then filtered through cotton. Sterilization is accomplished by autoclaving at 15 pounds' pressure for 20 minutes.

A piece of fresh beef is then obtained, and with as much precaution as possible to prevent undue contamination, a thin layer is removed, taking all of the exposed surface of the beef.[1] As much of the remainder as will be required is cut in small pieces and put through a meat chopper which has been previously boiled; 450 gm. of this ground beef and 10 gm. of dextrose are then added to every 1,000 cc. of the peptone solution. The flasks containing the medium are filled close to the cotton stopper in order to eliminate all the air space possible. The medium is then allowed to remain at refrigerator temperature for several hours, at the end of which time it is titrated against phenolphthalein and the reaction adjusted to +0.5, and is then sterilized by heating and maintaining it at a temperature of 65° to 70° C. for one hour on three successive days. It is then ready for inoculation.

The inoculation may be made either with a freshly isolated, virulent culture of the bacillus of symptomatic anthrax or with fresh affected muscle tissue known to contain only the blackleg organism. The latter method is not as easy of accomplishment as the first.

The culture that gives good results is one 24 to 48 hours old, recovered from a guinea pig which has been inoculated with virulent blackleg material. The cultures may be taken from the affected musculature, peritoneal fluid, or heart blood after the animal has been dead a few hours. Dextrose, beef, or liver bouillon may be employed in recovering

[1] This rejected material can be utilized in the preparation of ordinary beef bouillon.

the organism. A number of cultures should be made and care exercised to select for inoculation only a pure culture of the blackleg organism.

It has been the procedure of the writer to plan the work so that the culture would be ready for inoculation on the day the medium for toxin production was sterilized for the third time. The medium is allowed to cool down to approximately 40° C., and then several cubic centimeters of the culture are inoculated in the bottom of each of the flasks with a sterile pipette and the flasks placed in the incubator at 37.5°. If the inoculation is not made immediately following the third sterilization, the medium should be heated to 60° to drive off the oxygen and should be inoculated after it has cooled down to approximately 40°. Incubation for 10 to 12 days appears to be the approximate time for satisfactory toxin production.

The product is then removed from the incubator and filtered through several thicknesses of cheesecloth, next through a thin layer of asbestos wool, and then twice through Berkefeld filters of "N" porosity.

It is preserved with 0.5 per cent chloroform and stored in amber-glass bottles, which should be well filled, so as to leave as little air space as possible.

The product is tested culturally, and sublethal doses are administered to guinea pigs to determine whether or not it has been rendered free of organisms.

The potency of the material is determined through animal inoculation tests. In connection with the test for potency, attention has also been given to the degree of toxicity, because of the apparent relation of one to the other. This phase of the question will be given further consideration in a subsequent chapter of this article.

POTENCY TESTS OF THE TOXIC CULTURE FILTRATE

As in the case of the natural aggressin, numerous tests have been carried out with the culture filtrate on calves and guinea pigs. There is one important factor which has been uniformly noted in all the tests with the culture filtrate, and that is that there appears to be a direct ratio between the toxicity and potency of the product. In all potency tests thus far undertaken by the writer, no immunizing properties could be demonstrated in nontoxic culture filtrates. It is contemplated that if this relation of toxicity to potency is definitely proved, it will be a valuable factor in connection with standardization of the product.

Tables IV–VIII give results representative of the tests to which the filtrate has been submitted.

TABLE IV.—*Results of subcutaneous inoculation of guinea pigs [a] with the toxic culture filtrate, followed after 14 days with blackleg virus*

Guinea-pig No.	Amount of culture filtrate.	Amount of virus.[b]	Result.
	Cc.	*Cc.*	
1	0.25	0.5	Dead of blackleg in 36 hours.
2	.25	.5	Dead of blackleg in 48 hours.
3	.50	.5	Remained alive.
4	.50	.5	Do.
5	.75	.5	Dead of blackleg in 48 hours.
6	.75	.5	Remained alive.
7	1	.....	Died from the effects of the blackleg toxin before the inoculation of the virus.
8	1	.5	Remained alive.
9	Control.	.5	Dead of blackleg in 24 hours.
10	Control.	.5	Dead of blackleg in 30 hours.

[a] Guinea pigs weighing from 320 to 380 gm. were used. The minimal lethal dose (M. L. D.) of the blackleg toxin for guinea pigs of this weight was between 1 and 1.5 cc. when administered intramuscularly.
[b] The virus employed was prepared from affected muscle tissue in the manner described under Table I.

TABLE V.—*Results of subcutaneous inoculation of guinea pigs [a] with toxic culture filtrate, followed after 14 days with specially prepared blackleg virus*

Guinea-pig No.	Amount of culture filtrate.	Amount of virus.	Result.
	Cc.	*Cc*	
1	0.25	0.25	Dead of blackleg in 24 hours.
2	.25	.25	Dead of blackleg in 48 hours.
3	.25	.25	Do.
4	.25	.25	Dead of blackleg in 72 hours.
5	.25	.25	Remained alive.
6	.25	.25	Do.
7	.5	.25	Dead of blackleg in 48 hours.
8	.5	.25	Remained alive.
9	.5	.25	Do.
10	.5	.25	Do.
11	.5	.25	Do.
12	.5	.25	Do.
13	Control.	.25	Dead of blackleg in 24 hours.
14	Control.	.25	Do.
15	Control.	.25	Dead of blackleg in 48 hours.

[a] Guinea pigs weighing 320 to 380 gm. were employed. The minimal lethal dose of the blackleg culture filtrate for guinea pigs of this weight was 1 to 1.5 cc. when administered intramuscularly.

In tests of various blackleg immunizing agents on guinea pigs, difficulty has been experienced by most investigators with the type of virus employed for the infection of the animal subsequent to vaccination. Most workers have employed for the purpose emulsions of affected muscle tissue. The procedure usually followed is to weigh out a definite amount of ground affected muscle tissue, emulsify with a definite amount of physiological salt solution, filter through a thin film of cotton, and use a certain quantity of the filtered solution as a test dose. It is obvious that a test virus prepared in this manner would not be very satisfactory, especially when used on small animals such as guinea pigs. The number of blackleg organisms such material would contain would undoubtedly

vary greatly with the different amounts weighed. Depending on how well the material is emulsified, a greater or smaller number of the organisms would be left behind in the small particles of tissue which are filtered out. Foreign organisms frequently present in such affected tissue also are complicating factors when injected into the guinea pigs.

The writer has prepared a type of test virus which has given very good results in the guinea-pig tests and possesses a number of advantages over the emulsion of affected tissue. It is prepared in the following manner: Guinea pigs are inoculated intramuscularly with an emulsion of virulent blackleg tissue and usually die of the disease in from 24 to 48 hours. Cultures are then made from the guinea-pig carcasses into fermentation tubes containing dextrose bouillon. The culture medium is heated for approximately 10 minutes in the Arnold sterilizer just prior to inoculation in order to drive off the oxygen. It is allowed to cool down to about 45° C., inoculated, placed in vacuum jars, and incubated 24 hours at 37.5°. At the expiration of the incubation period the jars are removed from the incubator and all fermentation tubes showing evidence of good growth removed and examined for purity. The cultures are then thoroughly mixed in a crystallizing dish with sufficient lactose to make a soft paste, and this placed in a vacuum desiccator containing sulphuric acid. Care should be taken to protect the material from direct light by covering the desiccator with towels or by keeping it in a dark place. The material dries very rapidly. It is then removed and pulverized to a very fine powder in a sterile mortar and stored in wide-mouth amber-glass bottles at refrigerator temperature. When ready for use a definite amount of the powder is weighed out and taken up in a measured amount of distilled water.

The approximate minimal lethal doses of this virus for guinea pigs weighing 350 gm. can be established and this increased 10 times for a test dose in potency tests of blackleg products.

Virus thus prepared contains no foreign organisms, eliminating the possibility of complications in the animals inoculated with it; it is readily absorbed, and can be fairly accurately standardized. In this form the virus retains its virulence for a considerable time.

TABLE VI.—*Results of subcutaneous vaccination of calves with toxic culture filtrate followed after 14 days with intramuscular inoculation of blackleg virus*

Calf No.	Amount of culture filtrate.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	5	10	Slight swelling. Remained alive.
2	5	10	Very slight swelling. Remained alive.
3	Control	10	Dead of blackleg within 48 hours.
4	do	10	Do.
5	do	10	Do.
6	do	10	Do.

[a] The virus employed was an emulsion of affected blackleg muscle tissue.

The following table gives the results of a test conducted with two different specimens of blackleg culture filtrate. The specimen labeled "1" was demonstrated to be nontoxic for guinea pigs, while 1.5 cc. of the sample labeled "2" would prove fatal to guinea pigs.

TABLE VII.—*Results of subcutaneous vaccination of calves with blackleg culture filtrates No. 1 and 2 followed after 14 days with intramuscular inoculation of blackleg virus*

Calf No.	Blackleg culture Filtrate No.	Amount culture filtrate.	Amount of virus.[a]	Result.
		Cc.	*Cc.*	
1	1	5	10	Dead of blackleg within 48 hours.
2	1	5	10	Do.
3	2	5	10	Very slight swelling. Remained alive.
4	2	5	10	Do.
5	Control	None.	10	Dead of blackleg within 48 hours.
6	do	None.	10	Do.
7	do	None.	10	Marked extensive swelling. Animal recovered.

[a] The virus employed was an emulsion of affected muscle tissue.

The following test on guinea pigs tends to demonstrate an intimate relation between the toxicity and potency of blackleg culture filtrates.

TABLE VIII.—*Results of vaccination of guinea pigs with various culture filtrates followed after 14 days with inoculation of blackleg virus*

Culture filtrate No.	Approximate M. L. D. for 350-gm guinea pigs.	Amount vaccinated in each of 3 guinea pigs.	Dose of virus injected after 14 days.	Result.
	Cc.	*Cc.*	*Cc.*	
1	Nontoxic.	1	0.5	All 3 guinea pigs died of blackleg.
X	1.5	1	.5	1 of the 3 guinea pigs died of blackleg.
Y	2.5	1	.5	Do.
3	Nontoxic.	1	.5	All 3 guinea pigs died of blackleg.
Z	Nontoxic.	1	.5	Do.
5	4	1	.5	Do.

RELATION OF THE NATURAL AGGRESSIN TO THE TOXIC CULTURE FILTRATE

The question has often arisen as to the relation between blackleg natural aggressin and the toxic culture filtrate—that is, whether or not the immunizing principles in both products are identical. While the work conducted in this respect by the writer has not as yet been sufficient to draw absolutely definite conclusions, there is one factor which points to a distinct difference in the active principles of the two products.

Of the specimens of blackleg natural aggressin tested in this laboratory comparatively large doses of the material produced no symptoms of toxemia in the guinea pigs inoculated. The same specimens, however,

were found highly efficient in immunizing experiments. In the case of the culture filtrate the toxicity of the material was readily demonstrable and its potency appears to depend on its being toxic.

It is possible, therefore, that immunization with blackleg natural aggressin is brought about through the production of "antiaggressins," while with the toxic culture filtrate immunity is acquired through the production of antitoxin.

It is highly desirable that an entirely satisfactory and practical method of concentration of the toxic culture filtrate be obtained. Some of the methods which have been employed in the past are wholly or in part unsatisfactory, the toxin being either totally destroyed or its potency considerably lowered. As the toxicity of the culture filtrate is apparently related to its potency, obtaining the toxin in a pure or concentrated form would permit the production of an accurately standardized product of uniform dosage, based on the minimal lethal dose of the toxin for guinea pigs of a given weight.

In the filtration of the toxic culture some of the toxin is lost as a result of such process. It is therefore essential that the product be subjected only to such filtration as is necessary to insure the removal of all organisms.

It is hardly necessary to emphasize the economic importance of the toxic culture filtrate as compared with the natural aggressin, since the cost of its production is only a small percentage of that necessary to prepare the natural aggressin.

CONCLUSIONS

(1) Blackleg natural aggressin and toxic culture filtrate are highly valuable agents in immunization against symptomatic anthrax.

(2) Martin's peptone solution to which have been added ground beef and dextrose is best suited for the preparation of the toxic culture filtrate.

(3) The blackleg toxin is susceptible to such influences as air, light, heat, etc., and in order to insure a potent product measures should be employed to minimize its exposure to the same.

(4) There is apparently a direct relation between the toxicity of the culture filtrate and its potency.

(5) Virulent bouillon cultures of the bacillus of symptomatic anthrax to which lactose has been added and which are then dried and pulverized give very satisfactory results as a test virus in standardization tests of blackleg immunizing agents on guinea pigs.

(6) There is apparently a distinct difference between the immunizing principles in blackleg natural aggressin and blackleg toxic culture filtrate.

(7) A uniformly satisfactory and practical method of isolating or concentrating the blackleg toxin is highly desirable.

CONCENTRATION OF SYMPTOMATIC ANTHRAX (BLACKLEG) TOXIN

[PRELIMINARY PAPER]

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Following is a preliminary report on some experiments made for the purpose of devising a practical method for concentrating the toxin described by Kelser in the preceding paper. When received for concentration this toxin was a liquid obtained by filtering blackleg cultures through Berkefeld filters. Four cc. or more of this filtrate was a fatal dose for 350-gm. guinea pigs, killing within 48 hours when the dose was small(4–5 cc.) and in an hour if the dose was large (10 cc.). All of the toxicity tests in this report involve the intramuscular injection of the toxin or its concentrate into the leg of the experimental animal.

At first an attempt was made to precipitate the toxin with (1) alcohol, (2) half-saturation with ammonium sulphate, (3) saturation with ammonium sulphate, or (4) zinc chlorid. In general the chemical methods for the purification of tetanus toxin devised by Brieger and Boer [1] and by Hayashi [2] were followed, but without success. The precipitates obtained were not toxic to guinea pigs; the details of the chemical work will, therefore, be omitted. Foth [3] states (*p. 10, 21*) that the toxin can be precipitated out of a germ-free filtrate by an excess of absolute alcohol. Grassberger and Schattenfroh [4] in their monograph state that nontoxic products are obtained by drying the toxin *in vacuo* at 30° C. (*p. 21*) or by precipitation with ammonium sulphate or alcohol (*p. 24*).

The following method of drying the toxin to a paste which resembles the ordinary beef extract was successful on a laboratory scale. Experiments on large scale drying and on the keeping qualities of this product are under way. This method of drying has been applied by various investigators to the drying of meat, milk, and cultures. Among the first

[1] BRIEGER, L., and BOER. UEBER ANTITOXINE UND TOXINE. *In* Ztschr. Hyg. u. Infektionskrank., Bd. 21, Heft 2, p. 259-268. 1896.

——— ——— UEBER DIE TOXINE DER DIPTHERIE UND DES TETANUS. *In* Deut. Med. Wchnschr., Bd. 22, No. 49, p. 783–785. 1896.

[2] HAYASHI, H. WEITERE FORSCHUNGEN ÜBER DIE CHEMISCHE NATUR DES TETANUS-TOXINS. *In* Arch. Expt. Path. u. Pharmakol., Bd. 47, Heft 1/2, p. 9–18. 1901.

[3] FOTH, H. NEUE RAUSCHBRANDIMPFSTOFFE. *In* Ztschr. Infektionskrank. Haustiere, Bd. 10, Heft 1, p. 1–22. 1911.

[4] GRASSBERGER, R., and SCHATTENFROH, A. UEBER DAS RAUSCHBRANDGIFT UND EIN ANTITOXISCHES SERUM. 110 p. Leipzig und Wien, 1904.

to use this method was Shackell,[1] who in 1909 (*p. 336*) pointed out the application of the method to the drying of a relatively unstable toxin.

Into each of several 9-, or 15-, cm. petri dishes, 10, or 25, cc. of the filtered toxin were transferred. These were kept overnight in a refrigerator at −9° C. (16° F.). The dishes containing the frozen toxin were then transferred to Hempel desiccators containing sulphuric acid, one large or three small dishes to one desiccator. The desiccators were evacuated with a Geryk pump to 2 to 3 mm. of mercury and then transferred to the refrigerator at −9° C., where they remained until the contents of the dishes had dried to a paste. This generally took from 24 to 48 hours. It is probable that the drying of this toxin must be accomplished while it is frozen; a few attempts at drying *in vacuo* at room temperature resulted in complete loss of toxicity. To some portions of the toxin, which was strongly alkaline, a calculated weight of acid potassium phosphate (KH_2PO_4) was added for the purpose of ascertaining the influence of neutralization of the alkali on the keeping qualities of the toxin paste.

Numerous inoculation tests were made on guinea pigs, using the dried toxin dissolved in water. The tests indicate that there was little, if any, loss in toxicity. The typical blackleg condition was found in animals that had died 24 hours or more after intramuscular injection of a weight of toxin paste corresponding to one fatal dose of the original toxin.

It was shown by Grassberger and Schattenfroh[2] that blackleg toxin can kill very quickly, without macroscopic lesions. In this respect it differs from tetanus and other toxins, which kill rather slowly, after producing noticeable pathological changes.

[1] SHACKELL, L. F. AN IMPROVED METHOD OF DESICCATION WITH SOME APPLICATIONS TO BIOLOGICAL PROBLEMS. *In* Amer. Jour. Physiol., v. 24, no. 3, p. 325-340. 1909.

[2] GRASSBERGER, R., and SCHATTENFROH, A. OP. CIT., p. 17.

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